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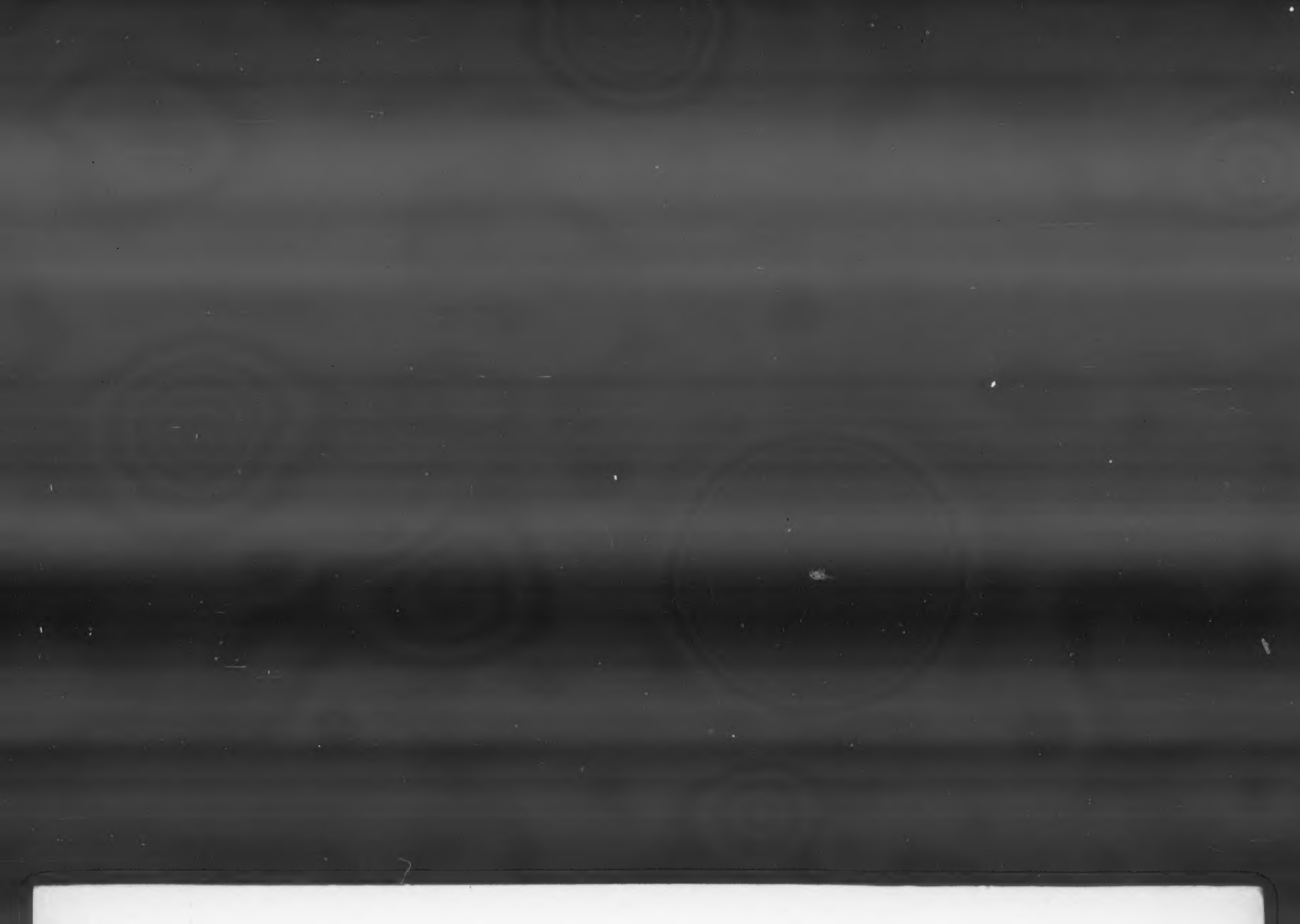

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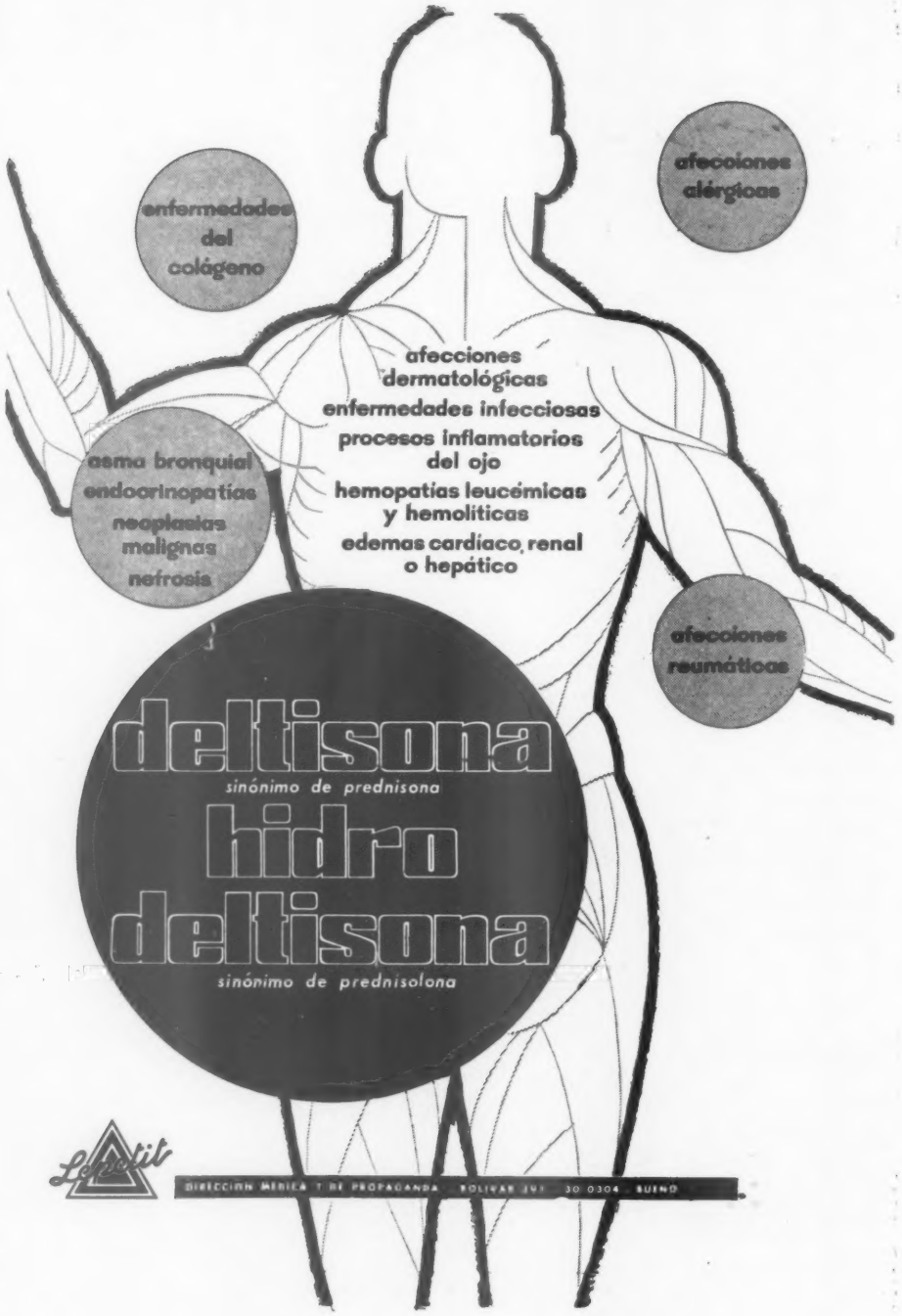
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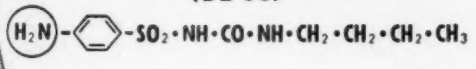

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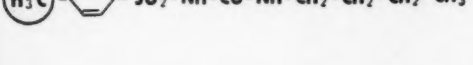

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ASOCIACION CIENCIA E INVESTIGACION

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ON THE ARTIFICIAL KIDNEY

I.- TESTING OF THE DISPOSABLE KOLFF-COIL KIDNEY

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INTRODUCTION

KOLFF ([2], [3]) has designed a new type of dialyser to be used in humans which has several advantages; it is disposable, requires only inexpensive components, is easily assembled and is simple to manipulate. It is efficient and has ultrafiltering capacity.

We have studied *in vitro* different values of "blood flow", bath fluid flow, outlet pressure, temperature and ultrafiltration capacity, in order to select the optimum conditions for work *in vivo*.

Our investigation was done according to the general technique recommended by Kolff ([2]).

Symbols. The symbols employed by Wolff ([5]) will be used in the description.

a: Volume of blood or other fluid circulating per minute in the artificial kidney.
A: Concentration of a substance in "arterial blood" (fluid entering the kidney).
B: Volume of bath fluid.
C: Clearance.
D: Dialysance.

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F: Volume of rinsing fluid circulating per minute in the artificial kidney.
R: Concentration of a substance in "venous blood" (fluid leaving the kidney).
S: Slope of the curve obtained by plotting the logarithm of A as a function of time.
T: Temperature of the rinsing fluid.
U: Concentration of a substance in rinsing fluid.
Uf: Volume of fluid ultrafiltered by the artificial kidney in unit time (rate of ultrafiltration of the kidney).
V: Volume of "blood" contained in the artificial kidney.
VP: "Venous pressure" (pressure registered in outlet tubes of the artificial kidney).

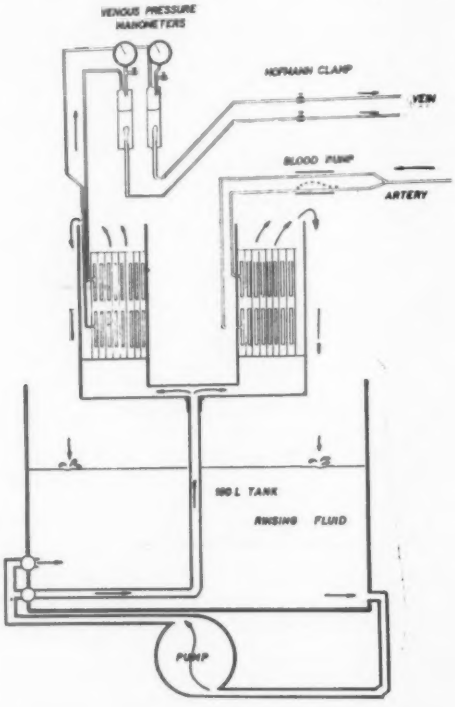

FIG. 1. — *Schematic diagram of the disposable, Kolff-coil kidney modified after Kolff et al* (2).

MATERIAL AND METHODS

The disposable Kolff-coil kidney (2) used was manufactured and supplied by Baxter Laboratories Inc. (Morton Grove, Illinois, U.S.A.). The installation and operation of the "kidney" was as outlined by

Kolff [2], and is shown schematically in figure 1. A Hofmann clamp was placed at the outlet tube of each coil in order to regulate the "venous pressure" VP.

In the following experiments, each coil was studied separately so that the results obtained with one might serve as a control for those of the other. Thirty liters of a solution containing 2 gm/l urea in water served as "blood". Half of the "blood volume" was pumped from the 30 l reservoir into each coil of the disposable unit and then through bubble catchers. In most experiments the fluid returning from the unit was discarded (open circuit system). In experiments measuring kidney volume and ultrafiltration and in studies performed *in vivo*, a closed circuit system was used. Each experiment was done in duplicate. Samples

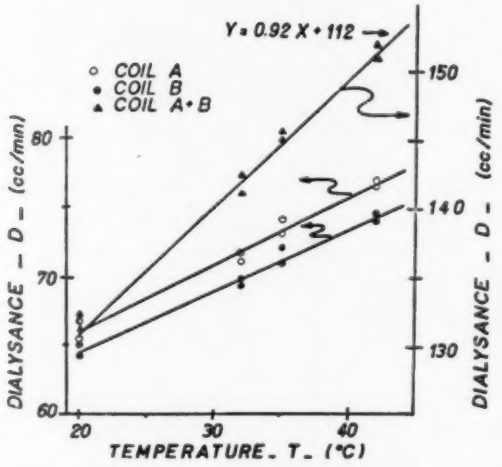

FIG. 2. — *Influence of temperature on dialysance.*

were taken from A and R. Urea analysis of each sample was done in duplicate by the Conway method [1]. The value of *a* was calculated directly by volume determination at the kidney outlet. In order to maintain the smallest concentration of urea in the rinsing fluid (U = 0) the latter (100 l) was changed every 20 minutes.

Clearance C was calculated and in our experimental conditions was equal to dialysance D [5]. If $C = \frac{a\,(A - R)}{A}$ and $D = \frac{a\,(A - R)}{(A - U)}$ then $C = D$ when $U = 0$.

EXPERIMENTS AND RESULTS

1. *Relation between temperature of bath T and dialysance D.* Flow rates and venous pressure were kept constant throughout this group of experiments at F = 8 l/min, a = 105 cc/min/coil and VP = 0 mmHg.

Temperature varied between 20° C and 42° C. Figure 2 shows that there is a linear relation between T and D. It may also be seen that the difference in results found between experiments done in duplicate with the same coil is insignificant. There was a consistent difference of 3 percent between the two coils of each kidney; this difference may be explained by position difference of the coils inside the artificial kidney. The total dialysance (addition of the dialysance of both coils) is a linear function of T. The slope of the line is 0.92 cc/degree and shows that under the working conditions of our experiment the dialysance

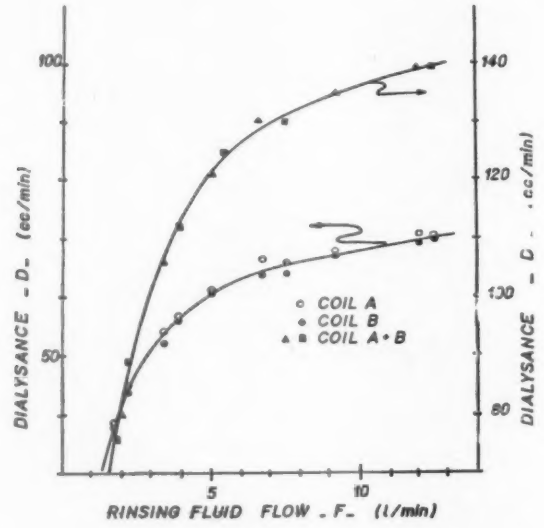

FIG. 3. — *Influence of the flow rate of the rinsing fluid on dialysance.*

varied approximately 1 percent with each degree of variation in temperature.

2. *Relation between the flow of the rinsing fluid F and dialysance D.* In these experiments arterial flow, venous pressure and temperature were kept constant throughout, a = 100 cc/min/coil, VP = 0 mmHg, T = 38° C. Flow of rinsing fluid varied from 2.0 to 12.4 l/min. Figure 3 shows a curvilinear increase in dialysance with increasing flow rate (F) with a rapid rise to approximately 5 l/min after which the slope becomes less steep.

It is apparent that flow should be greater than 5 l/min in order to avoid the large fluctuations in dialysance which occur with small differences in flow rate below 5 l/min. Flow rates of 8 l/min have therefore been used to get steady dialysances 10 percent greater than the obtained with F = 3 l/min, the flow rate recommended by Kolff [2].

3. *Relation between "blood flow" a and dialysance D.* Flow of the rinsing fluid, venous pressure and temperature were kept constant throughout the experiments: F = 8 l/min, VP = 0 mmHg, T = 38° C. "Blood flow" was varied between 30 and 210 cc/min/coil. Figure 4

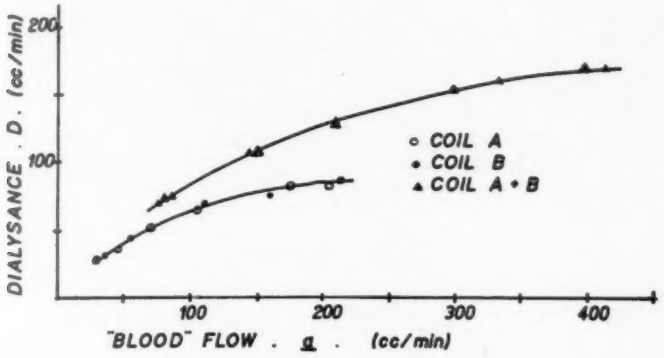

FIG. 4. — *Influence of the flow rate of "blood" on dialysance.*

summarizes the results. We shall limit our discussion to the curve obtained with the total dialysance (addition of both coils). It may be seen that the relation between *a* and D is curvilinear. The slope of the curve diminishes markedly with flow rates higher than 200 cc/min. For example: an increase of *a* from 100 to 200 cc/min increments D in

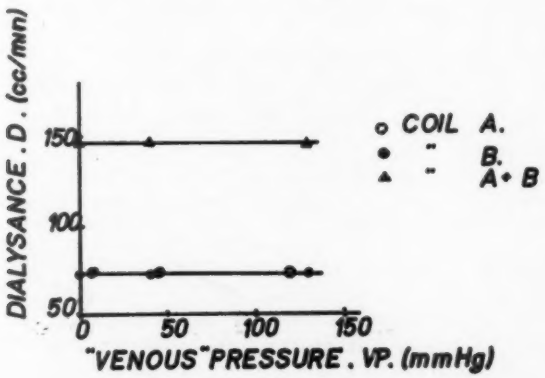

FIG. 5. — *Influence of venous pressure on dialysance.*

40 cc/min, while the increase of *a* from 200 to 300 cc/min raises D in 25 cc/min. From a practical point of view, dialysis in vivo should be performed with flow rate of about 200 cc/min. No significant advantages result from increasing *a* above 200 cc/min.

4. *Relation between venous pressure VP and dialysance D.* Flow rates and temperature were kept constant throughout the experiment at F = 8 l/min, a = 100 cc/min/coil, T = 38° C. Venous pressure was varied from 0 to 130 mmHg. Figure 5 shows that variations of VP do not influence O within the limits studied.

5. *Relation between "blood volume" V within the artificial kidney and variations in venous pressure VP.* A direct and precise measurement of the volume of blood contained in the artificial kidney (V) is not possible due to its structure. It has been reported that approximately 750 cc of blood are required for filling the artificial kidney ([2]); our experience is similar. An indirect method for measuring V was then used applying the formula described by Newman ([4]), S = C/V, in which S is the slope of the curve obtained by plotting the logarithm of the

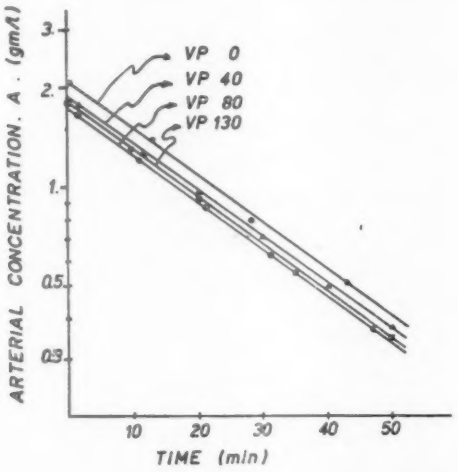

FIG. 6. — *Influence of venous pressure on the volume of "blood" filling the kidney. The abscissae is the time. The ordinate (semilogarithmic scale) represents the arterial concentration of urea. It may be seen that all the lines have the same slope.*

concentration of a substance in arterial blood as a function of time; C is the clearance of this substance, and V is the volume of distribution of the substance. It has been shown (experiment 4, figure 5) that variations in VP do not influence dialysance (D = C = constant = K). Newman's formula may, therefore, be expressed as S = K/V. Any change in V occuring during the experiments will be reflected in a change in S.

The experiments were performed as follows: flow rates and temperature were kept constant at a = 100 cc/min/coil, F = 8 l/min, T = 38° C. Venous pressure was varied between 0 and 130 mmHg. A solution of

2 gm/l urea in water was circulated through the kidney in open circuit. After a period adequate for stabilization, a closed circuit was established in such a way that the solution was taken from a reservoir containing 1000 cc urea solution, via the arterial tube, circulated through the kidney and was returned to the bottle through the outlet tube. Ultrafiltration loss was replaced by adding continuously water at a rate corresponding to that of ultrafiltration (calculated in a previous experiment similar to experiment 6). The circuit consisted then of a constant volume of 1000 cc contained in the bottle plus the volume (asumed to be variable) contained in the coils of the kidney. Samples of A were taken and the logarithm of the urea concentration plotted as a function of time (figure 6). The slopes found for VP of 0, 40, 80 and 130 mmHg were

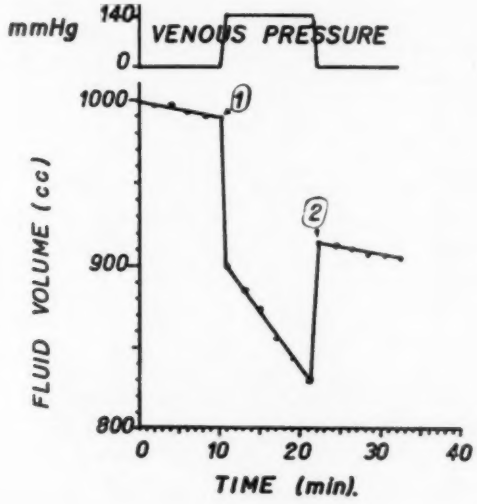

FIG. 7. — *Influence of venous pressure in the volume of "blood" filling the kidney. (See text).*

0.0318, 0.0325, 0.0330 and 0.0335 respectively. The differences are random and are within the experimental error. It may be concluded that variation of VP does not influence significantly V.

The former experiments were confirmed with a more direct measurement of the variation of V. With VP = 0, a = 200 cc/min (addition of the 2 coils), F = 8 l/min, a closed circuit was established with 1000 cc urea solution, in a graduated flask, at the beginning of the study. After the necessary period of stabilization, the Hofmann clamps at the kidney outlet were closed to a degree which raised VP from 0 to 140 mmHg. There resulted a decrease of 90 cc in the volume of the graduated flask. After a period of stabilization, the clamps were removed to return VP to 0 mmHg. The fluid level in the flask rose 90 cc. Figure 7 summarizes

this experiment. The abscissae represents time and the ordinate the volume of fluid contained in the flask (1000 cc at the beginning). It may be seen that from the onset until point 1, the volume decreases slowly. At point 1 (VP elevated from 0 to 140 mmHg) a sudden decrease of the volume equivalent to 90 cc occurs. For a few minutes the volume of fluid contained in the flask continues to decrease moderately. At point 2 (Hofman clamps released, VP = 0 mmHg) a rapid increase of 90 cc in the volume of fluid in the flask is found. It may be seen that the slope (corresponding to the ultrafiltration rate) between the beginning and point 1 is similar to the slope following point 2 (both ultrafiltration rates are equal). The slope between points 1 and 2 is greater; this is because of the increase in ultrafiltration rate corresponding to increment on VP.

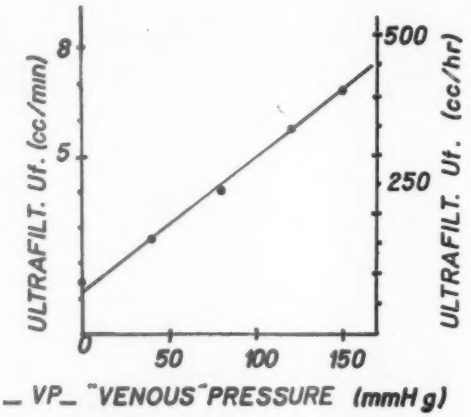

FIG. 8. — *Influence of venous pressure in the ultrafiltering capacity of the kidney.*

Experiment 5 indicate that a change in VP from 0 to 140 mmHg produces an increase of about 90 cc in the intrarenal volume. This value is not important in studies *in vivo*.

6. *Relation between venous pressure VP and ultrafiltration capacity of the kidney Uf.* A closed-circuit system was employed as in experiment 5. Tap water contained in a graduated flask was used as "blood"; it was also used as rinsing fluid. In this study urea was ommited from the system in order to avoid its osmotic effect. Flow rates and temperature were kept constant troughout the experiment at a = 200 cc/min (addition of the two coils), F = 8 l/min, T = 38° C. Venous pressure was modified from 0 to 150 mmHg. The decrease in water volume within the graduated flask, as a function of time represents the rate of the artificial kidney's ultrafiltration. Figure 8 shows that there is a linear relationship between Uf rate as a function of VP ($y = 2.25\,x + 67.2$ cc/hr). Ultrafiltration varying from 1 to 7 cc/min can be obtained by changing VP between 0 and 150 mmHg.

Experiment 4 showed that D is not influenced by variations in VP. Actually ultrafiltration should contribute to the elimination of urea from the "blood" but its contribution is a small fraction of D and therefore lacks of practical value.

7. *Relation between "blood flow" a and ultrafiltration Uf.* Venous pressure, temperature and flow of the rinsing fluid were kept constant at VP = 100 mmHg, T = 38° C and F = 8 l/min. A change in *a* from 60 to 420 cc/min (addition of the 2 coils) was made. Figure 9 demonstrates that Uf increases by only 50 cc/hr when *a* increases from 100 to 300 cc/min. Values higher than 300 cc/min have no apreciable influence on Uf. An increase in *a* from 100 to 200 cc/min raises Uf by 35 cc/hr, while increasing *a* from 200 to 300 cc/min raises Uf by only 15 cc/hr. It may be concluded that a = 200 cc/min is the optimal blood flow for ultrafiltration.

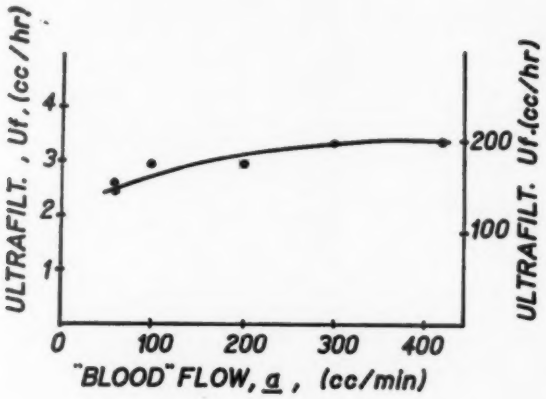

FIG. 9. — *Influence of the "arterial" flow rate on the ultrafiltering capacity of the kidney.*

8. *Ultrafiltration capacity Uf of the artificial kidney in vivo.* Uf was studied in 3 dogs in which ureteral ligation was performed. The dialysis was done using Kolff's technique and standard rinsing fluid ([2]) containing 4 gm/l glucose. Other losses being corrected or negligible, the weight loss of the dogs during dialysis was interpreted as loss of fluid by ultrafiltration. In each experiment VP of 30 and 150 mmHg were used, obtaining Uf of approximately 1.5 and 5.0 cc/min respectively. In one experiment, after a control period of 1 hour, the concentration of glucose in the rinsing fluid was raised from 4 to 10 gm/l. There was no important variation in Uf. No rupture of the cellophane coils was observed in spite of the variation in VP.

From the former studies it may be concluded that it is possible to vary the ultrafiltration capacity of the disposable Kolff-coil kidney by increasing the resistance at the outlet of the kidney (VP). This

occurs without significant change in either the internal volume of the kidney or in dialysance.

SUMMARY

A systematic series of experiments *in vitro* and *in vivo* was carried out for the purpose of studying the qualities and efficiency of the disposable Kolff-coil kidney. Dialysance and ultrafiltration were studied with varying temperatures, rate of flow of rinsing fluid, rate of flow of "blood" passing through the kidney and outlet pressure of the artificial kidney (venous pressure).

Temperature of the rinsing fluid has no important effect upon dialysance. The optimal rate of flow of rinsing fluid is approximately 8 l/min and the optimal rate of "blood" flow is about 200 cc/min. Increasing venous pressure raises the ultrafiltration rate without significantly increasing dialysance. There no increase in "blood volume" within the coils of the kidney with increasing "venous pressure". These qualities make the disposable Kolff-coil kidney an efficient instrument for dialysis and ultrafiltration.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Dr. W. J. Kolff for the valuable teaching given; to C. M. C. To Baxter Laboratories Inc. for their kind contribution of dialysing units and to Dr. V. Baracco and C. Vidal for their collaboration in the surgical aspects of this work.

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ISO - HEMODYNAMIC TRANSFUSION FOLLOWED BY SEVERE HYPOTENSION IN NORMAL DOGS

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THE IMPORTANCE of knowing whether or not natural blood incompatibility exists in the dog, is for the veterinarian and experimental physiologist quite obvious. So far the literature points out that no natural antibody is present in the blood of the dog, so that a single transfusion may always be performed with no concern for eventual danger to the organism. The experiments that will be described in this paper have shown, however, a marked discrepancy from previous studies on this matter, especially concerning the harmlessness of a single transfusion in the dog.

HISTORICAL REVIEW

The existence of immune antibodies in the dog, already pointed out as early as 1910 by von Dungern and Hirsfeld (1910) is beyond question. As far as natural blood incompatibility is concerned, the first paper to interpret as such some symptoms observed in the dog was by Ottenberg, Kalinski and Friedmann (1913). Of 22 transfusions described only 5 were performed in recipients not previously transfused; in 4 of them no symptoms appeared and in one transfusion the animal showed tremors of the hind legs, dyspnea and intense hematuria 24 hours after the experiment, with recovery after a few days. Similar results were presented by McEnery, Ivy and Pechous (1924) who found weak positive agglutination tests (titer from 1:2 to 1:6) in more than 2000 tests. From 9 transfusions they obtained two positive results (tremors, hyperpnea, chill, rise or fall of temperature). Olson (1940) found 41 per cent of positive agglutination tests when performed with plasma and practically none with serum, a fact, by the way, never confirmed afterwards. The number of transfusions is not stated, "several" expe-

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timen's made showed a wide variation of symptoms, from severe shock and death following convulsions and paralysis, to mild hypotension and hemoglobinuria. Hamilton (1949) contrary to the observations of all previous authors, found so clear out agglutination test as to guarantee classification of 90 per cent of the dogs in two groups, again a fact never confirmed. Wiener in his book about Blood groups and Transfusion (1943) does not even mention blood transfusion incompatibility in dogs, and denies a "distinct isoagglutination" with normal dog's blood. According to the papers of the Rochester School under the supervision of Young (1851, 1952) the demonstration of naturally occurring iso-antibodies is doubtful: "presumably attributable to differences in technique and possibly to confusion with non-specific cold agglutination and rouleaux formation". They found, however, in 15 per cent of normal dogs an extremely weak agglutination. As far as experiments "in vivo" are concerned they say: "Experience to date (1952) indicates that naturally occurring canine iso-antibodies, as well as canine anti-B, anti-C, anti-D and anti-E (immune antibodies described by them) in transfused dogs, are of little or no importance with respect to transfusion incompatibility".

Methods

Mongrel dogs were attached to the table in a dorsal position and anesthetized by intravenous Nembutal (25 mgrs per kilogram) and, if necessary, with additional small doses of Thionembutal. The external saphenous vein was cannulated; the arterial pressure taken from a cannulated femoral artery by a Ludwig manometer. A plastic tube with external end connected to a metal stopcock was inserted in the right auricle through the jugular vein and kept filled with heparinized saline in between the withdrawals of blood samples. The blood used for transfusion was taken from the jugular vein of non-anesthetized donors, and kept incoagulable by mixing it as soon as withdrawn with Heparin (0.13 ml of "Liquemin Roche" per 100 ml of blood, corresponding to 650 international units). The injection of the blood in the recipient dog was made through a small transfusion apparatus with a system of valves permitting aspiration of the blood from a container in a 37° C water bath, followed by injection in the saphenous vein, in the reverse stroke of the syringe. Transfusions were performed injecting 20 ml of blood in the cannulated saphenous vein and after a certain time allowed for mixing (30 seconds), a sample of the same amount of blood was taken from the right auricle. This procedure was repeated without interruption until the total amount of blood was injected into the recipient (usually from 100 to 200 ml). In this way the hemodynamic condition of the circulation remained practically undisturbed, since only small amounts of blood were withdrawn each time (Iso-hemodynamic transfusion). Hematological methods employed were described in a previous paper by Cruz, Silva and Pimenta Mello (1945). Heart and respiratory rates were periodically counted. Adenylic acid derivatives in plasma were determined by biological assay in the fowl caecum according to Barsoum and Gaddum (1935). The figures representing the measure of significance are the

multiples of the standard error of the difference of the proportions considered. Occurrence of hypotension has been compared with the standard proportion of 47 per cent, found as the result of 120 random transfusions (transfusions with dogs selected at random).

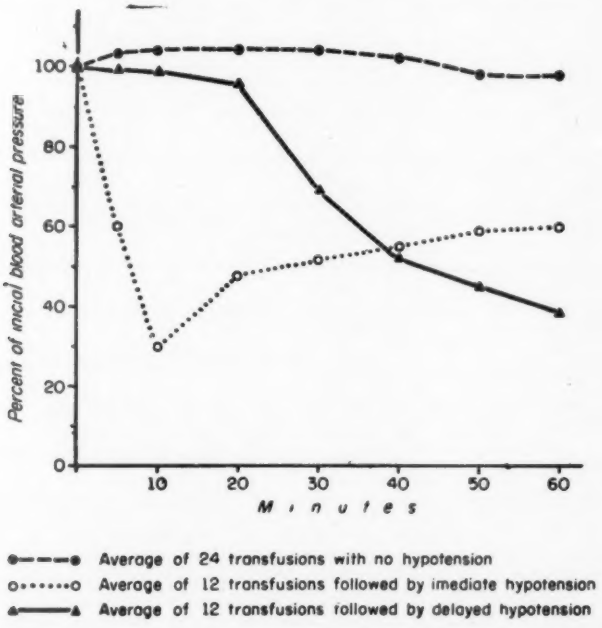
RESULTS

A. *Random transfusion experiments.*

1. *Dosage and rate of transfusion:* Three to four ml packed red cells per kilogram of body weight is the smallest amount needed to induce hypotension. The proportion of induced hypotension remains about the same (around 50 per cent) till 8 to 9 ml of packed red cells per kilogram; from this amount on the proportion increases to 70 per cent. The rate of blood injected varied between 6 to 48 ml per minute, most transfusions, however, were performed within the much narrower limits of 18 to 36 m. whole blood per minute.

2. *Abnormalities found after transfusion.*

a. *Blood pressure:* Hypotension is considered as such only when the blood pressure falls below 60 per cent of its initial value, during the first hour from the beginning of the transfusion. The results of 120



GRAPH 1. — *The difference of immediate and delayed hypotension is clearly shown in the average taken from 12 transfusions in each group.*

transfusions with various techniques are shown in Table I. The difference between transfusions followed by hypotension and those not, are very striking: 62 transfusions followed by hypotension showed an average of 48 per cent from the initial value (48 per cent i. v.), at the lowest arterial pressure observed, whereas in 67 transfusions not followed by hypotension a decrease only of 4 per cent (96 per cent i. v.) was observed. The observed hypotension could be divided in two groups: immediate and delayed, as shown in Graph 1 and 2. In 60 transfusions followed by hypotension, 27 were of immediate or intermediate type (45 per cent) and 35 of the delayed type (55 per cent). Two alternatives follow the fall of the arterial pressure: in some cases the pressure remains low for at least two hours, in others a more or less rapid recuperation, attaining almost

Average arterial

Transfusión	Permanence of blood before transfusion of washing	Nmber of dogs.	Initial arterial pressure (mm Hg).	Percentage of HYPOTENSION Minutes after transfusion										
				5	10	20	30	40	50	60	70	80	90	120
Whole blood	Room temperature 15' water-bath 37° C	29	117	84	81	68	58	50	45	44	46	49	54	56
	Constant stirring	4	107	78	74	55	66	60	56	62	65	67	70	—
Washed red cells	Washed immediately after removal	6	98	99	88	78	87	74	46	53	61	66	68	73
	30' room temperature	3	107	92	89	87	43	48	54	53	54	54	52	58
	15' water bath 37° C	5	136	96	89	67	54	49	45	41	36	30	36	47
	120' water bath 37° C	10	131	98	99	83	65	58	51	43	43	42	39	40
AVERAGE			116	91	87	73	57	56	49	49	51	51	53	55

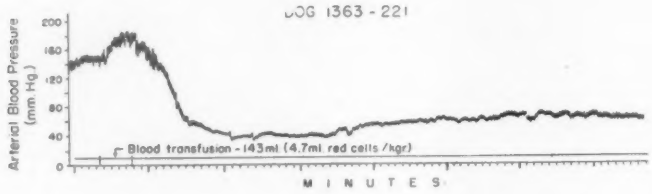
normal levels, is observed. The unrecovered cases go to a true shock, the venous oxygen saturation falling to a very low level; the recuperated cases do not show any apparent sequelae of the test, the animals being alert and gay the day after the experimental transfusion. The degree of incompatibility keeps good correlation with the recovery capacity of the arterial pressure: of ten dogs with low recuperative capacity (showing after 90 minutes 24, 25, 28, 29, 32, 32, 32, 37, 39 and 45 per cent i. v.), five died within 24 hours after the transfusion; whereas six dogs showing fair recuperation (after 90 minutes: 62, 67, 74, 78, 84 and 84 per cent i. v.) all survived more than 24 hours. To know if the selection of the correct donor was enough to induce hypotension in every instance, 11 donors which had induced hypotension once were used again two or three

determinations pressure

the initial arterial pressure															
		NO HYPOTENSION													
		Minutes after transfusión													
(1)	(2)	5	10	20	30	40	50	60	70	80	90	120	Total number of dogs.	Hypotension incidence (Per cent).	Multiple of the standard error of the difference between proportions.
32	122	105	103	104	105	102	100	99	92	89	88	81	61	47	—
3	129	96	97	98	99	98	96	96	—	—	—	—	7	57	0.52
6	116	99	102	105	101	101	102	99	87	75	83	—	12	50	0.20
7	138	98	101	193	103	102	97	86	79	77	76	71	10	30	1.03
6	140	86	86	84	84	83	86	82	78	70	65	69	11	45	0.13
9	136	95	104	106	105	98	96	88	79	77	76	77	19	53	0.49
	130	96	99	100	99	97	96	91	83	78	78	74	120	47	—

(1) Number of dogs.
(2) Initial arterial pressure (mm Hg).

times. Twenty three transfusions using this group of positive donors induced hypotension in 17 instances (75 per cent), a highly significant result (2.5 times the standard error of the difference) when compared with the standard proportion of 47 per cent of random transfusions. Besides



GRAPH 2. — *Immediate hypotension is observed sometimes as soon as the transfusion is finished. Of notice in this case is the very slight recovery after one and half hour.*

this, the absence of hypotension in 25 per cent of these transfusions showed that it is not enough to find the proper donor to induce hypotension, but that a suitable recipient has to be selected as well.

b. *Heart rate:* The heart rate remained normal throughout the experimental period in transfused dogs that did not show hypotension, but bradycardia was always observed in cases of transfusion followed by hypotension. Bradycardia could sometimes be very severe, in one case the heart rate fell, after 20 minutes from the beginning of the transfusion, to 60 beats per minute from an initial value of 168 beats. The most intense bradycardia observed (83 por cent from an initial value of 179 beats per minute), from 59 dogs showing hypotension, occurred 20 minutes after the beginning of the transfusion.

c. *Respiratory rate:* Increase in respiratory rate is observed in the hypotension or non-hypotension groups; the rate of increase, however, is much more pronounced when transfusion is followed by hypotension. In extreme cases, from an initial respiratory rate of 13 per minute, after 40 minutes, 24, 36 and even 68 respiratory movements per minute were observed, in marked contrast with cases not followed by hypotension in which, in some rare instances, a rate of 20 per minute was observed.

d. *Hematological data.*

Red blood cells: Both hematocrit and hemoglobin determinations showed that hemoconcentration occurred, as a rule, in most of the animals studied, independent of the occurrence of hypotension. Hypotension group: Initial hematocrit of 32 per cent and hemoglobin 10.9 gm per 100 ml of blood increased to 47 per cent and 13.5 gm after 60 minutes and 90 minutes respectively, as an average of 52 dogs. Non-hypotension group: Initial hematocrit of 34 per cent and hemoglobin 11.3 gm increased

to 47 per cent and 13.3 gm in the same periods of time, as an average of 52 dogs. To know if this hemoconcentration was induced by the blood injected or by anesthesia and prolonged attachment to the table, three control dogs were studied employing the same technique, but no transfusion performed; the absence of hemoconcentration in these animals showed that the hemoconcentration that follows transfusion is a side effect of the blood injected.

Hemolysis "in vivo": Only quite occasionally (7 observations), were traces of hemolysis detected by the naked eye (most of these long after the hypotensive crisis) in samples collected after transfusion from 116 observations in 28 dogs.

Hemosedimentation: The hemosedimentation results were parallel to those observed with hemoconcentration. The sedimentation rate decreased or was even abolished when the hematocrit increased (13 dogs of hypotensive group and 7 dogs with no hypotension). Even blood with a fast sedimentation rate (48 mm per hour) did not sedimentate after a rise of hematocrit from 40 to 61 per cent.

Leucocytes: Leucopenia is the rule in cases of hypotension. Three dogs with initial values of 13,500, 19,200 and 8,600 leucocytes per cu. mm, after 30 minutes showed respectively 1,800, 400 and 300 leucocytes per cu. mm. These figures increased somewhat afterwards as shown at 60 minutes: 2,400, 1,800 and 1,700 per cu. mm. Direct visual observation of the leucocytes layer in Wintrobe's tubes, after sedimentation followed by centrifugation of the blood, in samples taken from 20 dogs in which hypotension had been observed and from 6 dogs with no hypotension, confirmed these results.

e. *Survival rate:* From the hypotensive group 18 dogs died within 24 hours (25 per cent): one died on the table, one within four hours and 16 were found dead on the following day, whereas 55 dogs survived the first day, many of them recovering completely, acting normally several weeks after the transfusion. From the non-hypotension group all dogs survived more than one day (67 dogs).

B. *Transfusions performed with selected donors and recipients. Correlation Studies.*

1. *Blood groups obtained by immunization (hetero-agglutinins):* We have prepared anti-A serum checking its titer with a sample obtained from Rochester *. Sixty three iso-hemodynamic transfusions gave the following result: A+ donors with A+ recipients: 9 instances of hypotension in 19 transfusions (47 per cent); A+ donors with A− recipients: 5 instances of hypotension in 11 transfusions (45 per cent; 0.13 the standard error of the difference); A− donors with A+ recipients: 9 instances of hypotension in 14 transfusions (64 per cent; 1.16 s.e.d.); A− donors with A− recipients: 5 instances of hypotension in 9 transfusions (56 per cent; 0.52 s.e.d.).

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* We are grateful to Prof. L. E. Young (Rochester, New York) for the courtesy of sending a sample of anti-A serum.

2. *Weak natural agglutinins in the dog:* We have confirmed the presence of a weak natural agglutinin in the dog (titer 1:4 to 1:6). Cross-agglutination is the only technique to detect this incompatibility "in vitro" since clear-cut groups, such as existing in humans, are not found in dogs. Hypotension was observed in 12 instances with 21 transfusions between recipients having in their plasma natural iso-agglutinins to the red cell of the donor (57 per cent; 0.85 s.e.d.); whereas hypotension was observed in 8 instances with 15 transfusions in which natural agglutinins were absent (53 per cent; 0.44 s.e.d.).

3. *Fragility of the red cell of the dog in alkaline media:* This property of the erythrocyte of the dog, described elsewhere (11), is an individual particularity of this animal. According to the degree of fragility we divided the dogs into three groups: fragile, moderate and resistent. Combinations of these groups were made in 26 transfusions as follows: Donor-fragile and recipient-fragile (2 hypotension in 3 transfusions), recipient-moderate (2 in 2), recipient-resistent (1 in 3); Donor-moderate and recipient-fragile (2 in 2), recipient-moderate (3 in 3), recipient-resistent (2 in 6); Donor-resistent and recipient-fragile (0 in 1), recipient-moderate- (1 in 1) and recipient-resistent (2 in 4).

4. *Individual differences in reabsorption of red cells injected intradermically:* Skin red cell reabsorption test was prepared based on the wide discrepancy noticed in the reabsorption of blood injected into the skin of the dog: in groups of 10 dogs a 5 ml blood sample was taken from each dog, washed three times with saline and resuspended in equal parts of red cells and saline; 0.1 ml of these washed cells were injected intra-dermically in the abdomen and inguinal region, each dog receiving 10 injections of blood, one from each dog of the group. Twenty four hours later, a reading was taken comparing the amount of red cells still present in the site of each individual injection. Several lots of ten dogs were studied and 14 iso-hemodynamic transfusions performed: 11 transfusions with non-reabsorbed red cells showed hypotension in 7 instances, whereas 3 transfusions with easily reabsorbed red cells showed hypotension once.

C. *Blood incompatibility factor.*

Miscellaneous experiments: Taking the standard incidence of hypotension as 47 per cent (61 transfusions with donor's blod remaining at room temperature for about half hour) the following incidences were observed: 57 per cent (0.52 s.e.d.) when donor's blood was incubated at 37°C. during 15 minutes; 50 per cent (0.20 s.e.d.) when donor's blood was, as soon as removed, washed three times with saline and resuspended in saline before injecting into the recipient; 30 per cent (1.03 s.e.d.) when the blood was three times washed after being at room temperature for about 30 minutes. Total blood previously incubated during 15 minutes at 37°C. and then washed, gave an incidence of 45 per cent in 11 transfusions (0.13 s.e.d.), and incubated during two hours with identical technique gave, in 19 transfusions, an incidence of 53 per cent (0.49 s.e.d.).

Preventive substances: To know if hypotension was a consequence of acetylcholine or histamine liberation, dogs were injected previously to the transfusion with adequate amounts of atropine (1 mg atropine sulfate), and others with an anti-histaminic (Neo-Antergan, 2 ml corresponding to 50 mg of the active substance); neither substance was able to prevent hypotension.

Adenylic acid derivatives: To test the possibility of adenosine derivatives liberated from red cells causing hypotension with bradycardia, adenosine equivalent was determined in the plasma of one dog (arterial pressure falling from 114 mm/Hg to 48 mm; heart rate from 160 beats to 100 beats) with the following results: 10 minutes - 12 gamma per ml of plasma; 40 minutes - 15 gamma; 40 minutes - 12 gamma; 60 minutes - 5 gamma and 120 minutes - 12 gamma.

Discussion

The two different types of hypotension —immediate and delayed— seem to foster the speculation that no preformed substance of the donor's blood acts directly on the organism of the recipient, but an intermediate reaction going more or less slowly occurs between the injected blood and the recipient. If we take into consideration that hypotension occurs in 75 per cent of cases when donor's which had induced hypotension once were repeatedly used, it may be calculated that 65 per cent of random dogs might act as donor's to give the 45 per cent standard incidence in random transfusions.

Histamin, acetylcholine and adenylic acid derivatives are substances active in physiological processes apt to induce hypotension with bradycardia. Of these only adenylic acid derivatives are found in great amounts in the red cell. Deyrup (1951) found adenin derivatives when mammalian blood is mixed with hypertonic NaCl, but this treatment of the blood always resulted in hemolysis of various intensities. Studer, Fleisch and Croisier (1939) identified as adenosintriphosphoric acid the vasodilator substance from the red cells when hemolysed by distilled water. As no noticeable amount of hemolysis "in vivo", indicating breakdown of the donor's red cells, was ever found in dog's blood incompatibility, a mechanism connected with substances leaving intact the red cells would be the most probable explanation of the fact observed.

That hemoconcentration, sometimes very marked, may be detected in transfusions followed or not by hypotension, shows that the injected blood might, besides inducing hypotension, interfere in the distribution of plasma and cells, probably through a mechanism of spleen contraction which is allowed, in the dog, by the launching of a mass of red cells in the circulation.

The lack of correlation between the described incompatibility and natural or immune antibodies, or a particular fragility in the red cells of the dog (1957), or the ability of the skin to remove red cells after blood is injected intra-dermically, suggests a mechanism altogether different from other known incompatibilites. The consistent absence of hemolysis or hemoglobinuria in the recipient, are other facts indicating that canine incompatibility mechanism is rather of a biochemical nature,

while human blood incompatibility seems to be more of a cellular origin. Whatever the mechanism, however, the major role of the red cells transfused is beyond doubt, as carefully washed red cells incubated or not at 37° C for different periods of time, stirred at varying periods of standing before transfusion, give about the same incidence of the phenomenon.

The number of animals studied and the consistency of the results, when the test was many times slightly modified, seem to guarantee the existence of canine blood incompatibility. The practical importance of this finding is quite apparent in experimental physiology, where transfusion is a common resource of research workers in blood hemodynamics and shock, and cast doubt upon all contributions dealing with cross-transfusions or similar experiments.

Summary

Transfusions from 1/8 to 1/4 of the total blood volume were performed in dogs, in which care was taken to avoid disturbances of the hemodynamic equilibrium of the circulation. A small amount of blood was injected in the saphenous vein and after some time allowed for mixing, the same amount of blood was taken from a cannula inserted in the right auricle; this process was repeated until the total amount of blood was transfused.

Of 120 iso-hemodynamic transfusions in dogs not previously transfused, the blood was well received by the recipient in 53 per cent of the cases, but hypotension below 60 per cent of the initial arterial pressure, sometimes going as low as 20 to 30 mm Hg, was observed in 47 per cent of these experiments. Usually in both compatible and incompatible transfusions a more or less severe hemoconcentration was observed. Two types of hypotension were observed: one immediate, the arterial pressure falling within 10 to 15 minutes after the transfusion; another delayed, with the fall beginning after a latent period of 20 to 30 minutes. Both types of hypotension were accompanied by bradycardia and were followed by hyperpnea. In 25 per cent of positive hypotensive cases the animals died within the first 24 hours; others died later or survived in good condition; in these last cases the arterial pressure becomes normal within a two hours period after the transfusion. The blood incompatible factor is found in the red cell of the donor, since red cells washed three times and resuspended in saline were sufficient to induce serious hypotension similar to that observed with whole blood transfusion. The observed incompatibility was not correlated with blood groups observed in dogs by immunization; nor with the fragility of red cells of dogs towards alkaline media; nor with individual differences in reabsorbing red cells from other dogs when injected intra-dermically. Anti-histaminics or atropine do not abolish nor prevent the hypotension and adenosine equivalents are not present in excess in the plasma during or after the hypotensive period. The absence of hemolysis "in vivo", fever, chills, hemoglobinuria seem to point out that this type of dog blood incompatibility is of another kind than that described in humans. The actual impossibility of foreseeing by a reliable test this type of blood incompatibility interferes with the possibility of using cross-transfusions in studies

of shock and raises serious doubts as to the conclusions arrived at by these types of experiments in the past.

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BLOOD PRESSURE CHANGES AND BLOOD PRESSURE RESPONSES TO EPINEPHRINE AND LEVARTERENOL

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IT IS KNOWN THAT hypophysectomy produces a fall in blood pressure in amphibia ([1]), rats ([2], [3]) and dogs ([4]). It has been shown that total hypophysectomy ([5]) or neurohyphysectomy ([6]) in dogs results in a decreased resistance to hemorrhage, and that hypophysectomized animals are especially sensitive to the hypotension produced by histamine ([7]). This indicates a subnormal vasopressor response in hypophysectomized animals ([7]). However, the blood pressure responses to renin, hypertensin and epinephrine were normal in hypophysectomized dogs ([8]), and the blood pressure response to renin was increased in hypophysectomized rats ([2]).

The purpose of this communication is to report the results obtained in hypophysectomized turtles concerning the blood pressure changes and the alterations in the blood pressure responses to epinephrine and levarterenol.

METHODS

Male and female turtles *(Chrysemys d'Orbignyi)* of approximately 1.5 kg were maintained in special pools and were fed fish. The total hypophysectomy was performed by the method previously described in this laboratory ([9]). The control sham-operated animals were submitted to the same surgical procedure except that the hypophysis was not removed. In part of the sham-operated animals the hypophysis was completely exposed and in part the cartilage membrane covering the hypophysis was left intact. Since there were no differences in the blood pressure changes or in the blood pressure responsiveness they will be presented together.

The blood pressure was recorded by a mercury manometer, with a needle or plastic tube in the carotid artery. Heparin was used in the

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connecting tubes. The animals were maintained without general anesthesia and usually remained quiet during the procedures.

Epinephrine chlorhydrate * and levarterenol bitartrate ** were injected into the jugular vein by means of a plastic tube. The pressor substances were prepared daily, 2 μg/ml in a 0.7 % NaCl solution; doses of 0.2, 0.4, 0.8 and 1.6 μg were used.

It was observed that at low seasonal temperatures (below 12° C approximately) the blood pressure responses to epinephrine and levarterenol decreased. All the results presented here were obtained during seasonal temperature in which this seasonal variations did not occur.

The blood pressure responsiveness was studied comparing the results in three groups of hypophysectomized turtles (1, 14 and 28 days) with the corresponding sham-operated groups. The blood pressure changes were determined comparing the control blood pressure (before the operation) with the blood pressure during the determination of the blood pressure responsiveness, in the animals of the 1 and 14 days hypophysectomized and sham-operated groups.

Results

a) *The blood pressure changes after hypophysectomy.*

Twenty four hours after the surgical procedure there was a statistically significant decrease in the blood pressure of the hypophysectomized and sham-operated animals (Table 1). Expressed as percent the decrease was greater in the hypophysectomized group (28 %) than in the sham-operated group (15 %). In another group of turtles analyzed 14 days

TABLE 1

Blood pressure changes after hypophysectomy and Sham-operation.

Group	N	Mean blood pressure mm Hg	
		Before	After
24 h Hypophysectomy	10	47.0 ± 2.3	33.7 ± 2.1
		P = 0.0026	
24 h Sham-operation	9	50.2 ± 2.5	42.4 ± 1.5
		P = 0.0264	
14 d Hypophysectomy	10	53.5 ± 1.8	41.8 ± 2.0
		P = 0.0068	
14 d Sham-operation	10	52.2 ± 2.6	47.1 ± 3.6
		P = 0.2228	

* Clorhidrato de Adrenalina Geyer.
** Noradrenalina Byk.

TABLE 2

Blood pressure responses to epinephrine and levarterenol

GROUP	N	Increases in mean blood pressure - mm Hg							
		Epinephrine				Levarterenol			
		0.2. μg	0.4 μg.	0.8 μg.	1.6 μg.	0.2. μg	0.4 μg.	0.8 μg.	1.6 μg.
CONTROL	10	12.9 ± 1.3	15.8 ± 1.5	18.2 ± 1.0	22.6 ± 1.0	11.1 ± 9.9	15.0 ± 1.4	17.4 ± 1.5	20.2 ± 1.4
Sh.-op. 24 h.	10	8.3 ± 1.0	13.8 ± 1.4	16.8 ± 1.6	18.3 ± 3.0	7.8 ± 0.8	12.6 ± 1.4	16.8 ± 1.4	20.1 ± 1.6
Sh.-op. 24 d.	10	8.6 ± 1.7	12.3 ± 1.1	17.4 ± 1.3	21.6 ± 1.6	8.1 ± 1.0	12.5 ± 1.3	17.7 ± 1.9	21.3 ± 2.4
Sh.-op. 28 d.	6	11.3 ± 0.5	13.3 ± 1.0	15.3 ± 2.0	16.5 ± 1.0	10.3 ± 1.0	14.3 ± 2.3	17.2 ± 1.0	17.5 ± 1.8
Hyp. (−) 24 h.	9	10.5 ± 1.1	15.3 ± 0.7	17.5 ± 1.3	22.7 ± 1.7	8.9 ± 0.7	14.5 ± 0.7	18.7 ±0.6	21.3 ± 0.9
Hyp. (−) 14 d.	10	☆ 5.2 ± 0.8	☆ 9.3 ± 1.2	☆ 14.0 ± 0.9	☆ 18.1 ± 1.2	☆ 6.2 ± 1.0	☆ 10.6 ±1.3	13.8 ± 1.2	☆ 16.1 ± 1.1
Hyp. (−) 28 d.	10	☆ * 5.5 ± 1.0	☆ 9.2 ± 2.0	☆ 11.8 ± 1.3	☆ * 13.0 ± 1.0	☆ * 12.9 ± 1.3	☆ * 15.8 ± 1.5	☆ * 18.2 ± 1.0	22.6 ± 1.0

☆ $P < 0.05$ when compared with the control group.
* $P < 0.05$ when compared with the sham — oper. group.

after the operation, the sham-operated group showed only a slight decrease in the blood pressure, but in the hypophysectomized group there was a significant decrease, comparable with the changes occuring in the twenty four hour group.

b) *The blood pressure responses to epinephrine and levarterenol.*

The sham-operated groups (1, 14 and 28 days) and the 24 h. hypophysectomized group did not present significant changes in the blood pressure responses to epinephrine and levarterenol when compared with the control group (Table 2). However, the hypophysectomized groups (14 and 28 days) showed a statistically significant decrease in the blood pressure responses to the pressor substances.

Comparing the results of the hypophysectomized groups with the corresponding sham-operated groups, there was a significant decrease in the blood pressure responsiveness of the hypophysectomized animals only in the group in which the hypophysis had been removed for 28 days.

Discussion

The blood pressure decrease in the 24 hr hypophysectomized group was approximately the same as that of the sham-operated group. This fact suggests that the surgical trauma played an important role in the production of the low blood pressure in this animals. The persistance of the low blood pressure in the group in which the hypophysis had been removed 2 weeks previously, and the fact that there was only a slight decrease in the corresponding sham-operated animals, indicates that in the turtle, as in the other species studied, the hypophysis is necessary to maintain the blood pressure at its normal values. Further studies are necessary to determine if the low blood pressure in the hypophysectomized turtles is due to a corticoadrenal insufficiency, neurohypophysis insufficiency and/or metabolic disturbances.

The blood pressure responses to epinephrine and levarterenol were decreased in the 14 days hypophysectomized turtles, but the decrease was statistically significant only in the group in which the hypophysis had been removed for 28 days. It seems to be due to a gradual insufficiency of a mechanism that normally regulates the blood pressure responses to epinephrine and levarterenol. Failure of this mechanism may be one of the explanations for the low blood pressure in the hypophysectomized turtles.

Summary and conclussions

In turtles, *Chrysemis d'Orbignyi*, 24 hr after hypophysectomy there was a fall in blood pressure which was approximately the same in the hypophysectomized and the sham-operated group. Another group of hypophysectomized animals, analyzed 2 weeks after the operation, exhibited a significant decrease in blood pressure, whereas in the corresponding sham-operated group there was only a slight decrease in blood pressure.

Twenty eight days after hypophysectomy there was a significant decrease in blood pressure responses to epinephrine and levarterenol. However, in the groups in which the hypophysis had been removed for

1 and 14 days the differences in blood pressure responsiveness were not significant when compared with the corresponding sham-operated groups.

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DISTRIBUTION OF RADIOPHOSPHORUS IN BONE OF NORMAL AND OF DENERVATED LIMBS

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I) STUDY IN THE NORMAL ANIMAL.

IN ORDER to study the uptake by the bone of the phosphate ion labelled with P32, given subcutaneously or intravenously, to the *Bufo arenarum* H. (toad) and the influence of the denervation upon such uptake, the authors have begun by the study of a group of unselected animals. The results of their observations are summarized in this first part.

Instrumentation and technique.

A first group of 30 unselected female toads, whose weights ranged between 100 and 140 gr were injected s/c an isotonic solution of Sodium Ortophosphate (pH 7) labelled with radiophosphorus (P32), carrier free. Each animal received a dosis of 0.18 to 0.20 μC per gram of its weight, and has been sacrificed from 1 to 48 hours after the injection. Both tibia-fibula bones were removed from each animal, their wet weight measured, and digested with warm nitric acid. Each digest was finally diluted to 25 cc of solution.

The number of counts per minute of each solution was finally determined with a Geiger-Muller liquid counter, type M6M and a model 100C Panax Scaler.

The results of radiophosphorus uptake by the bone were expressed by two different methods: 1) in percentages, with respect to the amount of phosphate injected for each gram of tissue weight; 2) in percentages for each bone with respect to the total fixed by both bones in each animal.

Two groups of 5 toads each, were injected in the same manner, but previously to the injection of labelled phosphate, they were submitted to a simple surgical intervention consisting of the manipulation (without producing lesion) of the sciatic nerve on one side.

Results. — Figure 1 shows the results of 6 experiments, each one of them indicating a group of 5 toads. The experiments refer to the hours 1, 3, 6, 12, 24 and 48, after the injection and the values indicated in it

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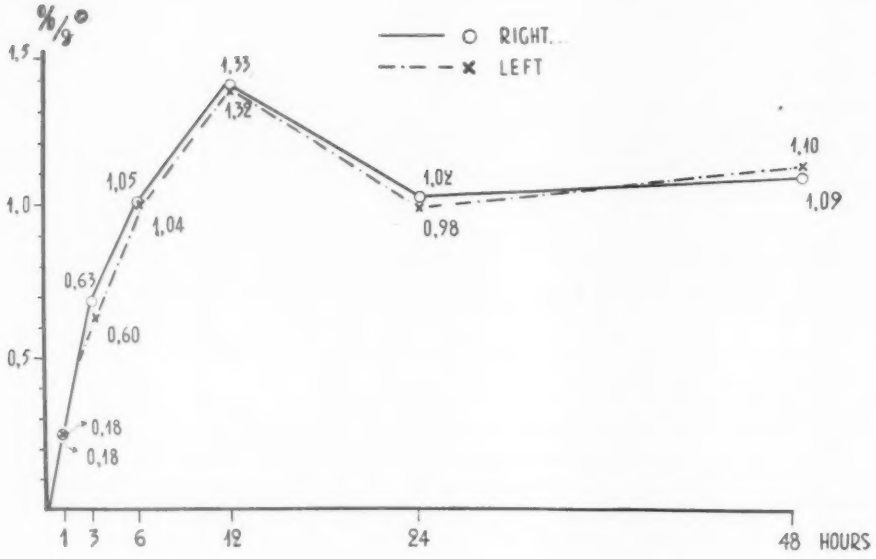

FIG. 1. — *Radiophosphate uptake by the tibia-fibula bones (right and left) in the* Bufo arenarum H. *The abscissae indicate: time in hours after the injection, Ordinates %/gr labelled phosphate fixed by the bones.*

are average fixations for right and left tibia-fibula bones. Graphs have been plotted on for the right-side, and another for the left-side bone.

A statistical analysis was made (test of "t") under the normal hypothesis that the tibia-fibula bones on one side (in this case the left side) fixed 50 % of the total amount captured by both bones; and it was discovered that the average of the individual values was 49.71 %. This value is not significantly different from 50 % and it allows us to accept the hypothesis of a symetric phosphate ion distribution between the left and the right tibia-fibula bones of the same animal.

In Table I are shown the summarized results of two short series of five animals each, which were operated with manipulation of the sciatic nerve one side, without producing lesion. It shows the uptake of labelled phosphate at the 3rd and 24th hours after the injection. The values of fixation were plotted in percentages with respect to the total fixed by both tibia-fibula bones, averaging 50.67 % (at the 3rd hour) and 49.05 % (at the 24th hour), for the bones of the treated side. These averages are not significantly different from 50 %, which serves as good reason to declare that the simple manipulation of the sciatic nerve is not a cause for variation in the uptake of the phosphate ion by the bone corresponding to the treated side.

II) STUDY IN ANIMALS WITH UNILATERAL SECTION OF THE SCIATIC NERVE.

Knowing the values of distribution and uptake of phosphate in both tibia-fibula bones of the normal animal, and having demonstrated that the radioactive phosphorus is distributed symetrically in both bones, regardless of manipulation of the sciatic nerve, the authors have studied a series of 25 unselected toads, whose sciatic nerve had been sectionated unilaterally at the upper point of the thigh and which were injected with labelled phosphate. The purpose of the study was to compare the uptake of radioactive phosphate in the intact side with the uptake in the other side whose sciatic nerve had been sectionated.

TABLE I

Labelled phosphate uptake in tibia-fibula bones of toads with manipulation (without resection) of the sciatic nerve.

1) Experiment Nº 13: 3 hours after injection.

		1	2	3	4	5	*AVERAGE*
T-F.B. Normal:	%	49.11	49.21	48.76	50.16	49.39	*49.37 %*
T-F.B. Manip:	%	50.89	50.79	51.24	49.84	50.61	*50.67 %*

2) Experiment Nº 7: 24 hours after injection.

		1	2	3	4	5	*AVERAGE*
T-F.B. Normal:	%	49.91	48.31	50.85	48.40	51.99	*49.95 %*
T-F.B. Manip:	%	50.09	51.69	49.15	51.60	48.01	*50.05 %*

T-F.B. Normal: Uptake % of tibia-fibula bone of normal side.
T-F.B. Manip. : Uptake % of tibia-fibula bone of the side whose sciatic nerve has been manipulated (without lesion).

Instrumentation and technique. — Same as mentioned in the first part of this paper.

Results. — In figure 2 the results of five experiments are shown, each one covering a group of five animals. The experiments correspond to 1½, 3, 6, 12 and 24 hours after injection, and the figures show the average values of fixation for tibia-fibula bones of the intact side and of the side which has been operated on (section of the sciatic nerve). In this figure the abscissae show the time elapsed (in hours) after the injection of phosphate, and in ordinates, the corresponding average fixation of P32 of the bone treated, for each group, expressed in % with respect to the total fixed by both bones.

This figure also shows the fixation in normal bones and in bones whose sciatic nerve has been manipulated.

Upon examination of the results obtained, it can be clearly deducted that the tibia-fibula bones of the side which has been operated on (upper section of the sciatic nerve) are fixing more radiactive phosphorus than the bones on the intact side. These differences, taken statistically, are

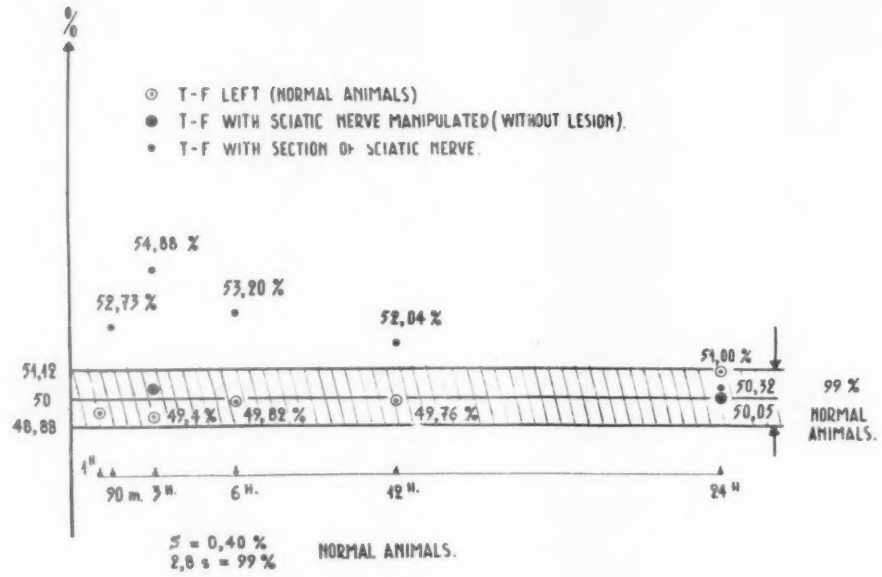

FIG. 2. — *Labelled phosphate uptake by the tibia-fibula bones of* Bufo arenarum *H. in normal tibia-fibula, in bones of animals whose sciatic nerve has been manipulated (without resection) and in leg-bones with the sciatic resected. The abscissae indicate: time in hours after injection. Ordinates: % of labelled phosphate uptake for each bone with relation to the total fixed by both bones of each animal.*

significant, reaching a maximum point at the 3rd hour after section of the nerve and injection of phosphate.

This maximum is followed by a progressive decrease at the 6th, 12th and 24th hour. For this last hour the values fail to show any significant variation.

Discussion. — The increased fixation of phosphate by the bones on the operated side is obviously an acute phenomena, which decreases after reaching a maximum point at the 3rd hour. This leads us to interpret this as a vascular phenomena. As a matter of fact, the resection of the sciatic nerve causes vasodilatation in the corresponding leg which can be observed in an increase of the skin temperature.

This vasodilatation by feeding larger amounts of radiophosphorus to the bone would be responsible for the observed variations.

III) STUDY IN ANIMALS WITH UNILATERAL EXTIRPATION OF THE LUMBO-SACRAL SYMPATHETIC GANGLIA.

Following the same technique a group of 5 toads was treated by extirpation of the lumbo-sacral sympathetic ganglia of one side. The results obtained show an increased fixation of labelled phosphate in the

bones of the denervated side, which would emphasize the hypothesis that an increased fixation is the result of phenomena of vasodilatation caused by the extirpation of the sympathetic ganglia or the resection of the fibers of the sympathetic which run in the stem of the sciatic nerve, along with the motor and sensitive fibers.

IV) STUDY OF THE UPTAKE OF LABELLED PHOSPHATE, ANALIZING BONES 30 DAYS AFTER THE DENERVATION.

In order to confirm that the increased uptake by the bones on the denervated side was due to an acute phenomena, the following procedure was followed: a group of 5 unselected toads was operated on by unilateral section of the sciatic nerve, and was injected weekly with labelled phosphate in the same doses as indicated above. This method assured a regular phosphate supply over a month. At the end of the month, the animals were sacrificed and readings were taken of both normal and denervated leg bones. The reading of the denervated leg bones indicated 50.47 % which is obviously a no-significant value. This confirmed that the process of chronic denervation has no influence on the uptake of labelled phosphate by the bone.

V) DISCUSSION.

In accordance with the studies of Weickel and others (1) referring to the mechanism of ion exchange at the crystalline surface of the bone (hydroxyapatite), it becomes easy to understand the reason why an acute process of vasodilatation is followed by an increase of the contact surface between the extracellular fluids and the crystalline phase of the bone. This explains the increased rate of exchange between the labelled phosphate ions fed by the extracellular fluids and the normal phosphate ions of the crystalline surface of the bone. As time passes on, in spite of this difference in the rate of exchange, a state of balance is reached in both bones (of the normal leg and of the operated leg). This does not outrule the possibility that the mechanism of uptake may be altered by the denervation, since our experiments refer to total phosphate, without discriminating for specific chemical fractions different from the phosphate fixed in the mineral bone.

SUMMARY

The authors have used labelled sodium ortho-phosphate to study the relationship between innervation and uptake of P32.

All determinations were made with a G.M. liquid counter type M6M and upon the tibia-fibula bone of *Bufo arenarum* H. (toad) previously digested with nitric acid.

In each animal the side operated upon (section of the sciatic nerve or removal of the lumbo-sacral sympathetic ganglia) was compared with the normal side. No natural assymmetry exists: a preliminary series of 30 animals was studied and no significant difference was observed between the right and left sides. Another control group was subjected to the same operative procedure except for the lesion of nervous elements: such animals exhibited no assymmetry.

After section of the sciatic nerve or after lumbo-sacral sympathectomy the animals were sacrificed, in groups of 5, at different intervals from the operation. The following results were observed: 1) each operation is followed by an homolateral increase in the fixation of P32; 2) differences between normal and denervated limbs increase gradually, reach a maximum 3 hours after the operation and then decrease; 3) 30 days later no significant differences subsist.

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DISAPPEARANCE FROM THE BLOOD OF SOME PENTOSES IN NORMAL DOGS UNDER THE INFLUENCE OF GLUCOSE AND INSULIN

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LEVINE ET AL. ([1], [2]) have shown that insulin increases the disappearence rate of certain sugars from the blood in the eviscerated-nephrectomized animal. These investigations give experimental support to the "permeability theory" of insulin action. According to this work the sugars having the same spatial configuration as glucose in regard to the first three carbon atoms will respond to insulin by increasing its distribution space in the body, as shown by the reduction in blood levels. Although the effect of insulin on the transport of sugars across the cell membrane has been repeatedly confirmed ([3], [4], [5], [6]), some doubts have been raised about the specificity of the transfer mechanism in relation to chemical configuration ([7], [8]). Stadie ([9]) has summarized the evidence, pointing out the fact that if the transfer theory appears correct in regard to the muscle cell, other cells may not behave in the same manner.

It would seem of interest to find a sugar different from glucose, not metabolized or slowly utilized whose disappearance rate would be increased under the influence of insulin in the intact animal under normal conditions. If such a sugar exists, its disappearance rate when injected simultaneously whith glucose might be assumed to be an indication of insulin secretion in response to glucose.

MATERIAL AND METHODS

Apparently normal dogs weighing between 13 and 19 kg, were used in this work. The dogs were previously trained with repeated venipunctures in order to eliminate fear and anger reactions. The animals were fasted overnight. In the morning of the test, a blood sample was taken from one of the veins of the limbs and immediately afterwards the sugar solution was injected within one minute using the same needle. At 15, 30, 45 and 60 minutes venous blood samples were withdrawn. These were divided in two portions, one for duplicate determinations of blood

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sugar according to the Nelson-Somogyi ([10]) and the other for duplicate estimations of blood sugar after fermentation using the same method. The sugars used: D-glucose, D-arabinose, L-arabinose, and D-xylose * in 3 M solution were injected at a dose of 1 ml/kg body weight. Each of the three pentoses were injected alone, with glucose, with insulin ** and with glucose and insulin **.

All the results were expressed in terms of "deltas" (differences from the initial values) in mg/100 ml. The statistical significance of the differences between means was appraised using the "t" test.

Results

The results are shown in Fig. 1 and Tables 1 and 2.

TABLE 1

Average and standard errors of the blood levels of non-fermentable reducing substances after the administration of different pentoses alone and in combination with glucose and insulin. (mg/100 ml)

	Nº experiments	Time in minutes after administration 15	30	45	60
D-arabinose	10	67±2	45±4	31±3	23±3
D-arabinose + Insulin	10	62±5	40±3	24±3	19±2
D-arabinose + Glucose	7	63±2	33±1 (*)	25±1	13±1
D-arabinose + Insulin + Glucose	7	54±5 (*)	30±3 (*)	23±4	15±2 (*)
L-arabinose	10	71±6	56±5	48±4	38±4
L-arabinose + Insulin	11	66±4	46±2	39±3	33±2
L-arabinose + Glucose	6	77±5	61±6	49±5	38±3
L-arabinose + Glucose + Insulin	8	69±4	49±4	37±4	30±3
D-xylose	10	61±3	51±3	43±3	35±3
D-xylose + Insulin	10	46±2 (*)	38±2 (*)	32±2 (*)	26±2 (*)
D-xlyose + Glucose	6	49±6	36±5 (*)	32±5	26±5
D-xylose + Glucose + Insulin	7	47±3 (*)	36±3 (*)	31±2 (*)	28±3

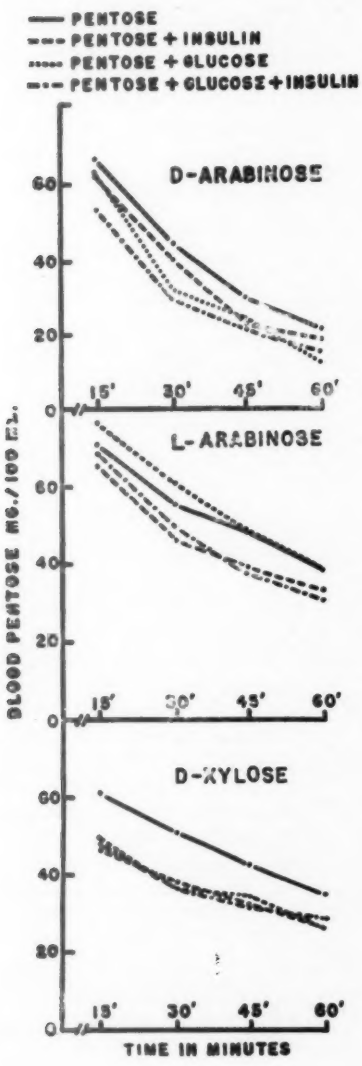
(*) Statistically significant differences in regard to the corresponding pentose injected alone ($t > 2$).

* Glucose Merk; D and L-Arabinose, Eastman Organic Chemicals; D-xylose Phansthiel Laboratories, Inc.

** Glucagon-free insulin, generously supplied by Eli Lilly.

Discussion

No difference was found in the blood disappearance rate of either D-arabinose or L-arabinose under the influence of insulin in the intact dog. The same was true of the action of glucose + insulin on the disappearance rate of L-arabinose. Glucose or glucose + insulin showed an influence on the blood disappearance rate of D-arabinose, probably related to glucose since insulin exhibited no effect. Blood levels of D-xylose decreased under the influence of insulin, glucose or glucose plus insulin and these decreases were of about the same magnitude. The effect of glucose on the D-xylose disappearance rate may be due to the extra insulin secretion induced by the former, but further experimental work on alloxanized animals is necessary to make this point clear. No explanation can be given at present about the effect of glucose on the disappearance rate of D-arabinose.



The finding of the ability of D-xylose to respond to insulin in the intact dog may be of interest to test the permeability effect of this hormone under chronic experimental conditions.

The findings in Table 2 suggest that D-xylose may not be metabolically inert in the intact animal since it produces a distinct rise in fermentable reducing power of the blood, which is presumably true glucose.

As the solutions of the pentoses used were of the same molarity it was possible to compare the differences in their disappearance rates. While L-arabinose and D-xylose did not differ significantly, both showed slower disappearance rates than D-arabinose in the normal dog.

Fig. 1. — *Average values of the disappearance of injected D-arabinose, L-arabinose and D-xylose from the blood on different experimental conditions.*

TABLE 2

Effect of pentose administration of blood fermentable reducing power ("true glucose"). Average of the differences from the initial values (mg/100 ml) and standard errors.

Pentose	Nº experiments	Initial value	Time in minutes after administration 15	30	45	60
D-arabinose	10	68±3	− 5±4	− 9±2	− 7±2	− 7±2
L-arabinose	10	71±3	+ 8±5	+ 6±5	0±5	− 2±4
D-xylose	10	73±4	+32±5	+18±3	+13±3	+11±3

SUMMARY

The influence of insulin, glucose or insulin + glucose, on the blood disappearance rates of D-arabinose, L-arabinose and D-xylose injected simultaneously were studied in the normal dog. It was found that insulin, glucose or glucose + insulin, accelerate removal of D-xylose from the blood. The effect of glucose may be due to induced insulin secretion. It this proves to be true, observation of the disappearance rate of D-xylose after the administration of this pentose together with glucose may be of use as an indication of insulin secretion. Further work in alloxanized animals is required. The influence of insulin upon blood xylose levels in the intact animal may be of value to study the action on the permeability effect of the hormone of factors influencing carbohydrate metabolism.

The disappearance of D-arabinose from the blood is increased under the influence of glucose or of glucose +-insulin, but no change was noticed under the action of insulin alone.

The removal of the individual pentoses from the blood was about the same for D-xylose and L-arabinose and faster for D-arabinose.

The injection of D-xylose leads to a distinct rise in blood fermentable reducing substances ("true glucose").

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CHEMICAL AND ANATOMICAL CORRELATIONS IN CHOLESTEROL-FED RABBITS

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SIMPLE FEEDING of cholesterol does not easily induce atherosclerosis in mammals, except in the rabbit and this has been widely demonstrated since the beginning of the present century (see early bibliography in [1]). The relationships between exogenous cholesterol and the development of atherosclerosis, though, are very complex even in the rabbit. Blood cholesterol levels (BCL), the amount of cholesterol deposited into the aortic wall, as well as the development of atherosclerosis increase with higher and longer cholesterol-feeding, but they also show a great variability among animals subjected to similar cholesterol-feeding procedures. The cause for this variability has not been totally clarified; in a very well controlled study, for instance, BCL even when roughly related to the amount and duration of cholesterol-feeding, showed a great scattering suggesting the influence of other factors besides variation in the amount of ingested cholesterol ([2]); that sex may partially explain this variability was demonstrated in the same paper since female rabbits showed higher levels and greater changes of blood cholesterol than male animals ([2]).

Following cholesterol administration the development of atherosclerosis too, is also very irregular. Thus, in a careful report, when 39 rabbits were given 1 g cholesterol/day during 90 days and the aortic lesions graded from 0 to 4, 30 % of the animals had 0-1 graded aortas ([3]). Furthermore in the same study no strict relationship was demonstrated between the intimal lesions and the amount of chemically determined cholesterol present within the aorta ([3]). The overall picture will conceivably become still more complicated if arterial lesions in *different* territories are correlated with chemical changes in the blood and in the vessels themselves.

Even if in man no strict correlation apparently exists between exogenous cholesterol and atherosclerosis ([4]), it seemed important to

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study the effect that cholesterol levels may bear to the development of atherosclerosis in several vascular beds in a species reportedly prone to this lesion under such experimental conditions. Consequently, the following data were determined in control and in cholesterol-fed rabbits: 1) blood cholesterol; 2) aortic cholesterol; 3) macroscopic atherosclerosis in the aorta, and 4) microscopic coronary lesions.

Material and methods

Sixty two male rabbits, 1.6 to 2.3 kg of weight on a standard laboratory diet including vegetables and added vitamins were weighed weekly (these animals are those remaining from a larger initial group; animals which died before being sacrificed have been omitted from the present study). Forty eight animals were given in addition, 1 g cholesterol daily, except Sundays in 5 ml of sunflower seed (I.N. 130) thoroughly mixed with mashed carrots. From this group and twice a week 15 rabbits were given intravenously 0.2 mg of β-estradiol benzoate; 11, 2 ml of 6 % dextran and 9, the same amounts of dextran and estradiol benzoate two hours apart. The results which were obtained in the last three groups of rabbits were similar in all respects and they will consequently be considered together; the experiment consisted then of 14 control and 48 cholesterol-fed rabbits.

After 90 days, the animals were anesthetized with pentobarbital, blood was withdrawn from the aorta and a complete autopsy was performed. The lesions in the aorta were drawn on a paper and "blindly" graded from 0 to 4 plus according to the extent and thickness of the lesions; the heart was fixed in 10 % formaldehide, cut into six slices parallel to the A-V grove and sectioned at 10 μ; in each segment 10 random sections were stained with Sudan IV-Hematoxylin and all arteries greater than 30 μ and clearly showing the different wall structures were graded as follows (tangential sections were disregarded): grade 0.5, initial endothelial and/or subendothelial infiltration of isolated sudanophilic granules; grade 1.0, medium endothelial and/or subendothelial infiltration of sudanophilic confluent granules; grade 1.5, marked endothelial and/or subendothelial sudanophilic infiltration forming a total or partial arterial obstruction; grade 2.0, inicial endothelial and/or subendothelial cellular proliferation of isolated cells; grade 2.5, medium endothelial and/or subendothelial cell proliferation forming small plaques; grade 3, marked endoethelial and/or subendothelial cell proliferation forming partial or total obstructions with or without superimposed degenerative changes. In order to quantitate the results, the individual lesions in each of the six heart segments were multiplied by the following factor: XI, if they numbered 1 to 5 different lesions as defined above; X2, if they numbered 6 to 10, etc. By this procedure we tried to prevent computation of a single lesion as if it were more than one. All the results thus obtained were added in the different segments and a total grade for the heart was thus obtained.

Blood cholesterol levels were determined according to the method of Bloor ([5]). The cholesterol content in the aorta was determined as follows: the arch was preserved for histological study; the adventicia of the rest

of the aorta was stripped and the remaining tissues extracted in boiling alcohol for 8-10 hours; cholesterol was determined in the alcoholic extract.

RESULTS

Blood values. — (Table 1). Blood cholesterol levels averaged 63 ± 5.3 mg/100 ml in the control rabbits and 679 ± 51.3 mg/100 ml in the cholesterol-fed rabbits.

TABLE 1

Cholesterol levels in the blood and in the aorta of control and cholesterol-fed rabbits.

Group	Number of rabbits	mg/100 ml Blood cholest	Aorta cholest. mg/100 mg wet tissue
Control	14	64 ± 5.3	0.23 ± 0.01
Cholesterol-fed	46	679 ± 51.3	0.80 ± 0.02

* Standard deviation of the mean $s = \sqrt{\frac{d^2}{n(n-1)}}$

Cholesterol content of the aorta. — (Table 1). Control rabbits had 0.23 ± 0.01 mg cholesterol/100 mg wet tissue in the aorta. Cholesterol-fed animals had values of 0.80 ± 0.02 ranging between 0.3 and 1.76 mg/100 (See graph Nº 1).

Aortic macroscopic changes. — (Table 2). None of the control rabbit showed intimal lesions in the aorta. In cholesterol-fed animals, 11 showed 0 graded aorta; 8, grade 1; 9, grade 2; 14, grade 3, and 5, grade 4 (one aorta was inadvertedly lost).

Coronary microscopic lesions. — In 40 rabbits, the coronary lesions found in 60 sections of the 6 heart segments were plotted against those present in 10 sections of the two higher cardiac segments and straight relationship was obtained for grades greater than 4 in the more detailed study; in other words, if 10 sections are reviewed only minimal atherosclerosis can be missed; consequently, as the results are not greatly changed,

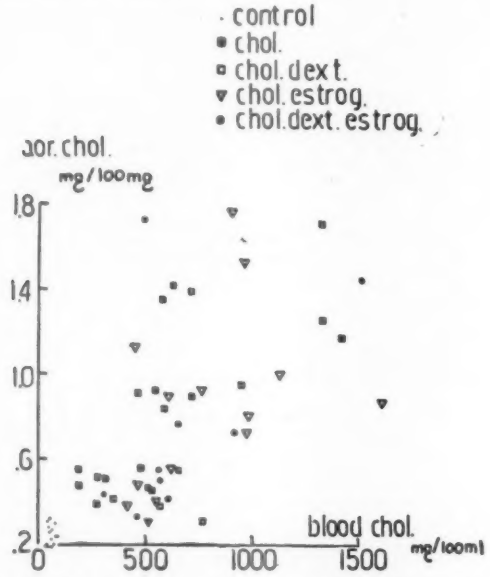
TABLE 2

Aortic macroscopic lesions in control and in cholesterol-fed rabbits.

Group	Number of rabbits	Aortic lesions-grade				
		0	1	2	3	4
Control	14	14	—	—	—	—
Cholesterol-fed	47	11	8	9	14	5

in the remaining rabbits we have studied only 10 sections of the two higher heart segments and the results refer to this method.

In the 14 control rabbits, intimal proliferation without lipid infiltration (grade 2.0 and 3.0) was found in 2 hearts, the remaining were normal. In the 48 cholesterol-fed animals, lesions were found in 35 hearts ranging from 0.5 to 79 (See graph Nº 2).



GRAPH 1. — *Aortic cholesterol content against blood cholesterol levels. All cholesterol-fed rabbits are randomly distributed. Chol., cholesterol; dext., dextran; estrog., β-estradiol benzoate. Discussed in text.*

Correlation between blood cholesterol levels and weight changes. — No correlation was found between blood cholesterol levels and body weight changes.

Correlation between blood cholesterol levels and arterial changes. — (Table 3).

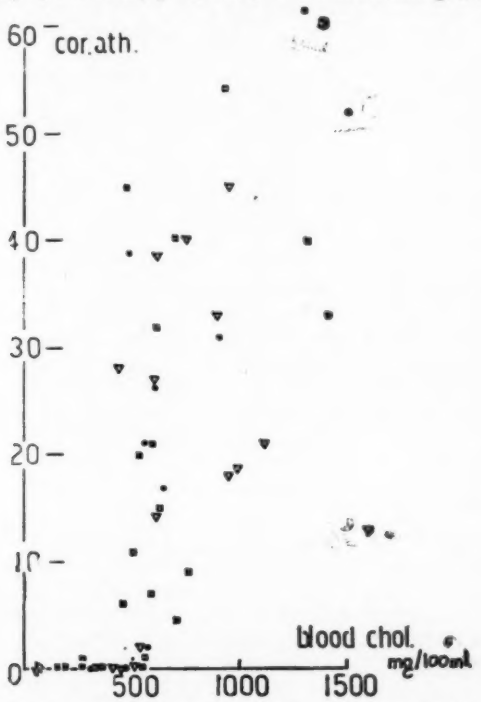
a) Aortic lesions. A rough relationship ($r = 0.70$, $p < 0.01$ *) between blood cholesterol levels and the development of aortic lesions is clearly seen in graph Nº 1. It should be observed, though, that a great overlapping of the results is present and that a few rabbits may show no atherosclerosis with cholesterol levels five times as high as normal while some animals may show advanced intimal changes at similar BCL.

b) Coronary lesions. An increased incidence of coronary atherosclerosis is present with higher cholesterol levels as shown in graph Nº 2. This correlation is not a straight one due to the great scattering of

* r, correlation coefficient; p, probability, accordingly with Fisher's tables (13).

the individual points ($r = 0.48$; $p < 0.01$). Futhermore, it is apparent that under present experimental conditions coronary lesions appear at BCL higher than 450 mg/100 ml and a "threshold" for coronary atherosclerosis may thus be defined in the rabbit; such a "threshold" was not demonstrated for the aortic wall.

c) Cholesterol in the aorta. The cholesterol deposited into the aorta increased roughly ($r = 0.55$; $p < 0.01$) with increasing BCL in spite that



GRAPH 2. — *Coronary atherosclerosis against blood cholesterol levels. Symbols as before. Discussed in text.*

a wide scattering of individual point was also present (See graph Nº 1); some aortas may show then a very high cholesterol content at moderate hypercholesterolemias and viceversa, may not show a greatly increased cholesterol content at markedly high BCL.

Correlation between aortic lesions and its cholesterol content. — That increased severity of the aortic lesions is present when the cholesterol content of the aortic wall increases, is apparent in graph Nº 3. This correlation is very close ($r = 0.91$, $p < 0.01$) although in a few cases no macroscopic lesions may appear even when the cholesterol content is 3 times as high as normal; contrarywise, any degree of aortic lesions may be present with similar total cholesterol contents.

TABLE 3

Chemical and anatomical correlations in control and in cholesterol-fed rabbits. Values of the correlation coefficients. The correlation coefficients must be considered to be only a first approximation because of discontinuity in the anatomical grading.

	Aortic cholest.	Coronary atherosel.	Aortic atherosel.
Blood cholesterol levels	0.55 *	0.58 *	0.70 *
Aortic atherosel.	0.91 *	0.79 *	—

* Statistically significant at the level of $p < 0.01$.

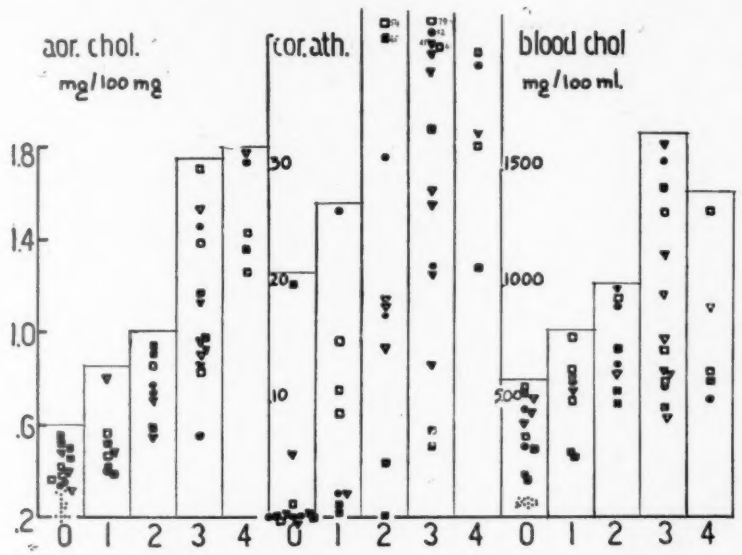
Correlation between aortic and coronary atherosclerosis. — When atheromatous changes were compared in the aortic and in the coronary arteries, it was apparent that a rough correlation between both existed ($r = 0.79$; $p < 0.01$) although here again, great scattering of the individual data is present (See graph Nº 3). In other words, some rabbits may show advanced coronary changes with absent or minimal aortic atherosclerosis and viceversa, marked aortic involvement with scanty coronary lesions.

Discussion

Our results confirm many previous experiments showing that in the rabbit hypercholesterolemia, increased cholesterol content of the aortic wall, and atherosclerosis follow the ingestion of cholesterol. Nevertheless, it is demonstrated that these parameters, although mutually interdependent, are not closely correlated.

Blood cholesterol levels (BCL) have shown great variability even when the animals have been placed under similar experimental conditions as far as the feeding of cholesterol is concerned *. Ingestion has not been measured but apparently played no part since no correlation was found between weight changes and BCL. A similar variability in the BCL has been recently reported by Fillios and Mann in a very careful experiment ([21]); we have eliminated some of the factors which enhance variability of the BCL, such as sex ([2]), since we have studied only male animals. Nevertheless, it is evident that mechanisms involved in the absorption, excretion, transformation and deposition of exogenous cholesterol must be understood in order to explain changes in BCL as observed under present experimental conditions.

* It must be stressed that in the present experimental results showed a random distribution when the different substances injected are considered; higher dosages of intravenous es radiol benzoate when combined with dextran injections, prevent atherosclerosis in cholesterol-fed rabbits (unpublished observations).



GRAPH 3. — *Aortic cholesterol content, coronary atherosclerosis and blood cholesterol levels against aortic macroscopic atherosclerosis. Symbols as before. Discussed in text.*

Aortic macroscopic changes differed greatly within the group of rabbits fed similar amounts of cholesterol. For instance, 19 of our 47 cholesterol-fed animals showed none or minimal aortic atherosclerosis; this point has been sometimes overlooked and may explain conflicting results when experiments involving few animals are discussed. In a paper by McMillan, Horlick and Duff ([3]), it is stressed that one third of rabbits fed 1 g cholesterol a day during 90 days may be considered as resistant to the development of aortic atherosclerosis and our results fully agree with their conclusion. Why some rabbits develop atherosclerosis and others do not, is not presently known. Our experience shows that only a rough correlation exists between BCL at death and the development of arterial lesions. Although BCL at death may not be representative of the conditions prevailing during life since weekly changes have been reported under conditions similar to the present experiment ([2]) it is evident that this determination may provide a standard of comparison, but cannot totally explain the degree of aortic involvement. A similar lack of clear correlation between BCL (and other blood lipids too) and atherosclerosis has also been demonstrated in human necropsies ([6]).

The aortic lesions can not be explained as a simple reaction toward cholesterol deposited within the vascular wall although it must be admitted that a correlation between both parameters exists. It was

evid nt that any grade of arterial involvement could be present at corresponding cholesterol contents and in a few instances the cholesterol content was greatly elevated and no macroscopic lesions were present. Similar conclusions have been advanced by McMillan et al [3]. This again points to other mechanisms which, when understood, will possibly allow to clasify the arteries as "hyper-", "normo-" or "hyporeactive" toward the cholesterol deposited in the arterial wall (similar mechanisms may possibly be involved toward substances other than cholesterol). Furthermore, the demonstrated lack of a linear correlation between BCL and cholesterol content of the aorta clearly means that cholesterol is not passively deposited in the arteries; as a fact this substance must follow the same hemodynamic laws as in the rest of the organism, namely, be ultrafiltered from the intima (and from the vasa vasorum) and reabsorbed from the venous capillaries of the vasa vasorum and the lymphatics (incidentally, if lymphatics play a similar role in the absorption of lipids as in the intestines, they may be important in the genesis of atherosclerosis, a question seldom raised in this connection [7]). In the arterial tissues, other mechanisms too, such as synthesis, local destruction, phagocytosis and removal may also contribute to the balance of local deposition and it is thus understandable that the amount of cholesterol present in the aortic wall may not parallel BCL. Such a conception is opposite to the one formerly held by Weinhouse and Hirsch who stated "...that the intima is freely permeable to the lipids of the plasma and that the intimal walls exerts no selective action on these lipids" [8]. This view which has gained popularity was arrived at by comparing chemically determined lipids in intimal extracts from 25 routine autopsies with plasma values obtained by Page et al, using different methods on a selected group of healthy men, i.e., those with total lipids between 400 and 650 mg/100 ml [9]. It is interesting to point out that shortly afterwards the same authors performed in rabbits an experiment where blood levels were compared with chemical determinations in the aorta of the same animals and rightly concluded that "...tissue factors not clearly understood at this time are important in determining the sites of lipid deposition" [10]. Now, twenty years later, we still ignore the factors involved in the deposition of cholesterol into the arterial walls as well as those involved in the reaction of the arteries toward this deposited cholesterol.

Coronary arterial lesions also showed a great variability within our cholesterol-fed rabbits and even animals with a normally low BCL have shown spontaneous intimal proliferation. The presence of coronary lesions, then, although grossly related to the BCL cannot be totally explained through such a mechanism since the individual observations were greatly scattered, i.e., some rabbits showed pronounced coronary atherosclerosis with moderately elevated BCL and viceversa, only moderate atherosclerosis with pronounced hypercholesterolemia. Here too, the coronary arteries behaved as if being "hyper-", "normo-" or "hyporeactive" toward BCL. Furthermore, it is interesting to point out that under our experimental conditions a "threshold" for the development of coronary atherosclerosis existed since animals with BCL below 450 mg/100 ml had practically no coronary lesions. In this respect, the coronary arteries and the aorta were different because an aortic

"threshold" was not evident. Reasons for this disparity deserve further study.

Finally, lesions in the coronaries and in the aorta were only roughly interrelated, this correlation not being a strict one. Some rabbits had pronounced coronary atherosclerosis with macroscopically normal aortas and viceversa, greatly atheromatous aortas could be found in rabbits with practically normal coronary arteries. The fact that these two vascular beds may be relatively independent and also that lesions are not strictly correlated with BCL, points again to the importance of local arterial factors in the genesis of atherosclerosis.

SUMMARY

In 14 control and in 48 cholesterol-fed rabbits, the following parameters were studied: 1) blood cholesterol levels; 2) aortic cholesterol content; 3) macroscopic aortic atherosclerosis, and, 4) microscopic coronary atherosclerosis.

Cholesterol-fed rabbits after 90 days showed hypercholesterolemia, increased aortic cholesterol, macroscopic aortic atherosclerosis and microscopic coronary atherosclerosis, but a great variability in the observed results was evident. By correlating the different observations it is concluded that unknown factors, other than the amount of ingested cholesterol determine blood cholesterol levels and that the concentration of this substance in the blood only partially explain atherosclerosis or vascular cholesterol deposition. The aortic and coronary lesions although roughly correlated, showed in some instances a relative independence. The importance of local and general factors in the genesis of atherosclerosis is thus stressed.

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PERIPHERAL VASCULAR REACTIVITY IN NORMAL DOGS AND IN DOGS WITH ADRENAL ATROPHY PRODUCED BY RHOTHANE (DDD) (*)

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THE ACTION of vasoactive substances under the influence of some hormones has been studied in our laboratory in an attempt to find out the role of peripheral vascular reactivity in arterial hypertension. The action of the thyroid gland [1] on the vascular response of rats and the changes caused by total and partial hypophysectomy in turtles [2, 3] were thus demonstrated.

The influence of the adrenal gland as a modifier of the pressure response of some substances was also studied. While most authors find a decrease of the response in adrenalectomized animals [4, 5], others do not find changes in the reactivity [6].

The contradictory results obtained in adrenalectomized animals as well as the importance of these findings led us to study the action of Rhothane (DDD) on the peripheral vascular reactivity. As this compound causes a gradual atrophy of the adrenal cortex, it might help in finding out the difference of responses in animals and man [7].

Since Nelson and Woodward (1949) reported that 2,2-bis(parachlorophenyl)-1.1-dichloroethane (DDD) produced the atrophy of the adrenal cortex in dogs, a systematic investigation of all the cortex functions has been carried out in order to determine whether they were altered [8, 9].

MATERIAL AND METHODS

Nineteen dogs weighing from 6.5 to 12 kg were used and their normal reactivity was determined. Rhothane was administered to 18 dogs, of which 12 were used to establish the relation between the dose and the time necessary to bring about symptoms of adrenal insufficiency.

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* This work was presented at the First Meeting of the Latin American Association of Physiological Sciences (Punta del Este, Uruguay, 1957).

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Finally, the vascular response to adrenaline, noradrenaline and acetylcholine was determined in the remaining group of six dogs before and after receiving DDD.

Once the animal was anaesthetized with nembutal (30 mg/kg), the femoral artery was cannulated and the blood pressure was recorded with a mercury manometer on a smoked cylinder.

TABLE I

Peripheral vascular reactivity in normal dogs and in Rhothane treated dogs, under the action of adrenaline, noradrenaline and acetylcholine

Number	Condition	Adrenaline µg 5	10	15	Noradrenaline µg 5	10	15	Acetylcholine µg 5	10	15
4	Normal	+ 34	+ 42	+ 60	+ 42	+ 38	+ 62	− 46	− 58	− 47
	Rhothane (12 g)	+ 24	+ 58	+ 56	+ 36	+ 54	+ 62	− 28	− 49	− 50
9	Normal	+ 21	+ 28	+ 50	+ 34	+ 55	+ 61	− 47	− 65	− 60
	Rhothane (22 g)	+ 6	+ 20	+ 21	+ 18	+ 24	+ 30	− 48	− 50	− 55
10	Normal	+ 45	+ 62	+ 72	+ 52	+ 90	+ 100	− 28	− 45	− 56
	Rhothane (65 g)	+ 48	+ 37	+ 44	+ 34	+ 45	+ 60	− 61	− 73	− 74
12	Normal	+ 17	+ 59	+ 66	+ 40	+ 55	+ 75	− 46	− 54	− 54
	Rhothane (10 g)	+ 21	+ 31	+ 46	+ 42	+ 55	+ 67	− 30	− 38	− 36
13	Normal	+ 30	+ 36	+ 37	+ 38	+ 63	+ 65	− 53	− 54	− 45
	Rhothane (10.5 g)	+ 15	+ 34	+ 40	+ 34	+ 48	+ 48	− 33	− 51	—
15	Normal	+ 30	+ 49	+ 54	+ 36	+ 28	+ 45	− 71	− 80	− 68
	Rhothane (7 g)	+ 27	+ 39	+ 36	+ 33	+ 52	+ 82	− 26	− 30	− 24

The vasoactive substances were injected through a vein of the leg. All the animals were previously heparinized and a sodium citrate solution was used as anticoagulant in the manometer connections.

Single doses of 5, 10 and 15 micrograms of adrenaline, noradrenaline or acetylcholine * were injected with intervals of 2 minutes between each dose and 5 minutes between each substance. Rhothane ** was administered to the dogs in gelatine capsules at feeding time and the doses varied from 31 to 176 mg/kg/day during a period of 8 to 57 days.

* Geyer adrenaline, Byk noradrenaline and Roche acetylcholine.

** Rhothane was supplied by the laboratories of Rohm and Haas Company, Philadelphia, Penn., U.S.A.

RESULTS

The modifications of the vascular reactivity in Rhothane treated dogs can be seen in Table I. It can be observed that under the experimental conditions used there was a certain variability of response either in the normal animal or after the animal was given Rhothane. In male dogs 4, 12, 13 and 15 the response obtained under the action of Rhothane with adrenaline and noradrenaline is smaller for most of the doses in-

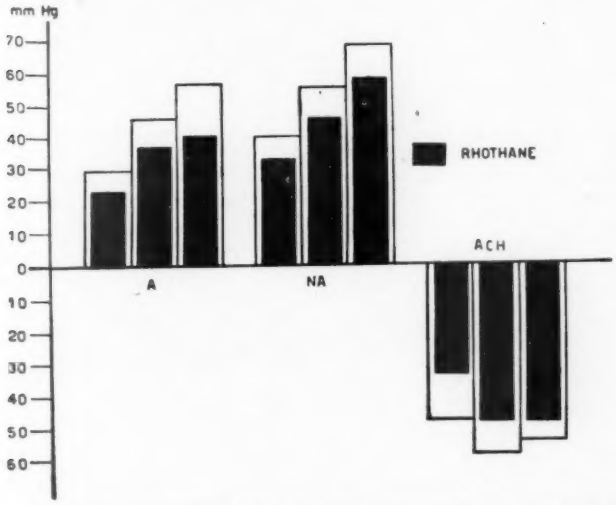

FIG. 1. — *Action of adrenaline (A), noradrenaline (NA) and acetylcholine (Ach) in the dosis of 5, 10 and 15 μg on the vascular response of normal and Rhothane (DDD) treated dogs.*

jected than in normal animals. In the case of acetylcholine, there was a less pronounced, but more constant decrease in depressor response. In female animals 9 and 10 an acute decrease of pressor response to adrenaline and noradrenaline was noted in all the concentrations used. In the case of acetylcholine a slight decrease of depressor response in dog Nº 9 and a great increase in dog Nº 10 was observed.

In spite of these individual variations, it can be seen (Fig. 1) that with the different doses of substances used, the average values were smaller for those dogs receiving DDD, thus demonstrating a decrease of vascular response. A progressive increase of pressor response with increasing doses was obtained in the normal dogs as well as in those receiving Rhothane.

Table I shows that the vascular response is directly linked to the dose and the time of administration. Thus the animal (dog 9) presenting the smallest response was the one receiving the greatest total dose during the longest period of time (44 mg/kg/day during 57 days).

Modifications in the initial arterial pressure were not usually observed in the period during which the animals were under the action of Rhothane. In those dogs belonging to the group used to establish the relation dose/administration time and which received a greater quantity of Rhothane per kg of weight, a decrease of pressure was sometimes noted, during the determination of reactivity. Recovery was impossible in most cases, thus causing the animals death.

TABLE II

Relation between body weight (BW) and weight of the adrenal glands (AW) in normal and Rhothane treated dogs*

Normal dogs BW	AW	Number	Rhothane treated dogs Dose	BW	AW
kg	mg		g	kg	mg
6 to 10	1.600	16	8.5	6	440
8 to 10	1.750	3	13.0	8.5	780
		4	12.0	8.4	1.280
		15	7.0	8.8	770
10 to 12	1.900	1	14.0	10	1.170
		2	14.0	10.3	1.263
		12	10.0	11.5	928

* Houssay and Molinelli, 1926 ([10]).

Table II shows the considerable decrease in weight of DDD treated glands as compared with the values for normal glands reported by Houssay and Molinelli ([10]). Histological observation showed atrophy of variable extent, depending on the administered dose and on the time of treatment. Cytotoxic alterations appeared in the first stages. These were localized in the fascicular and reticular zones and consisted in the appearance of big vacuoles in the cytoplasm and of nuclear pycnosis.

The fascicular and reticular zones underwent later a fragmentation of the cellular trabeculae accompanied by extensive lesions of necrobiosis (dog 12).

Finally, the glomerular zone was also altered, as it occurred in animals with more severe lesions (dogs 4 and 15) (Fig. 2), in which all the cortex cells presented pycnotic nuclei and cytoplasm desintegration, as well as congestion of the sinusoids and exudation of leucocytes. In some cases, the lesions reached the medular cells in the boundary with the cortex.

The dogs receiving DDD present the following symptoms during the period of observation: loss of appetite, muscular leanness, apathy

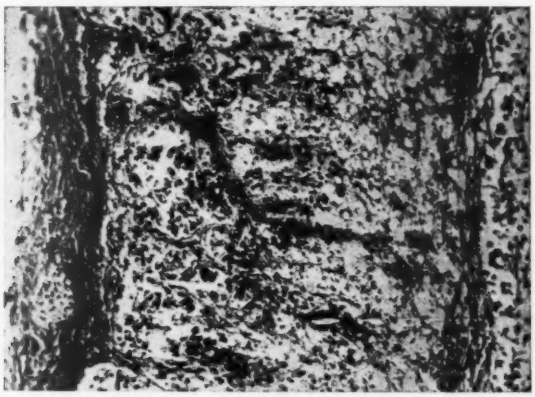

FIG. 2. — *Histological aspect of the Rho hane treated adrenal gland (dog 4): atrophy and cortical necrobiosis (x 240).*

and a tendency to infections with slow cicatrization. In the majority of dogs under observation the appearance of edema without change of weight was noted.

DISCUSSION

The influence of the adrenal gland over the vascular response was demonstrated by Chambers and Zweifach ([11]) using rat mesoappendix as well as by other authors ([5]).

They found that the cortical hormones play an important role in the maintenance of the vascular tone. When these hormones are lacking, the terminal vascular system loses its functional activity progressively, the vasomotion comes to a stop with an acute alteration of the precapillary sphincters. These functions are reestablished by the administration of corticoids ([12]).

Up to the present time all the observations about the role played by the adrenal gland in the vascular reactivity have been made by means of surgical adrenalectomy. This method has been probably responsible of the contradictory results obtained by the different authors, since in some instances the sensibility of the vascular response may be modified

as a result of alterations in the sympathetic vasomotor system ([6]). On the other hand, the difficulty of keeping the adrenalectomized animal alive for some time, does not allow to make a thorough observation during the experience.

The majority of authors ([4]) who tried to establish a relation between the adrenal gland and the vascular response found a decrease in the pressor response only when the adrenalectomized animal reached a stage in which the arterial pressure could not be maintained and fell progressively, as it happened in some of the animals we used in our experiments directed to establish a relation between the dose and the time necessary to induce symptoms of adrenal insufficiency.

Our work was, however, carried out using an animal with chronic adrenal insufficiency, which allowed us to make a thorough study of the different stages of the gland atrophy. Thus it was possible to work with an animal in good general conditions, which did not present either modifications in the initial arterial pressure or symptoms indicating shock.

As to the differences of response between the adrenalectomized animal and man with adrenal insufficiency ([7]) we believe that the cause of this discrepancy lies on the different conditions under which these experiments were carried out. It was observed that according to the dose of DDD used and the time of administration, several degrees of insufficiency could be established which corresponded to a smaller or greater decrease of vascular response. This fact was entirely confirmed through the histological study of the gland.

Finally, in view of the results obtained it would seem convenient to study further the role played by the sexual difference.

SUMMARY

The peripheral vascular reactivity was studied in normal dogs and in Rhothane (DDD) treated dogs using vasoactive substances such as adrenaline, noradrenaline and acetylcholine.

Different degrees of adrenal insufficiency were established according to the dose used and the time of administration which induced a decrease in the vascular response. This fact was confirmed through histological studies.

The results obtained by different authors are discussed and a parallel is established between surgical adrenalectomy and the pharmacological adrenalectomy brought about by DDD. It is shown that the latter process presents advantages when it is desired to study the differences of the responses obtained in the animal and in man with adrenal insufficiency.

Finally, and according to the results obtained, the convenience of a further study of the role played by the sexual difference is indicated.

ACKNOWLEDGEMENTS

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PRIMER CONGRESO NACIONAL DE CIENCIAS FISIOLOGICAS

(México, 23-25 enero, 1958)

Diferencias de acción entre la digitoxina y la ouabaina. J. ACEVES * Y R. MÉNDEZ. *(Departamento de Farmacología, Instituto Nacional de Cardiología, México, D. F.).*

Doctrinalmente, no se admiten otras diferencias entre los glicósidos cardíacos que aquellas que se refieren a su absorción, acumulación y rapidez de acción y eliminación. Sin embargo, algunos cardiólogos muestran preferencia por determinados glicósidos en las diferentes afecciones cardíacas. Así, Chaves y su escuela, por observación clínica, insisten en que la digitoxina ejerce acción más intensa que la ouabaína sobre la frecuencia sinusal y el sistema de conducción aurículo ventricular por lo que prefieren la digitoxina para el tratamiento de la fibrilación auricular.

Nuestros experimentos en perros anestesiados con pentotal cloralosa demuestran que, con la misma dosis, referida a tanto por ciento de la dosis letal, la digitoxina y la ouabaína presentan las siguientes diferencias:

Primero: La digitoxina ejerce un efecto bradicardizante más pronunciado que la ouabaína como resultado de acción vagal refleja más intensa y de diferencias en su efecto directo sobre el nodo seno auricular.

Segundo: la digitoxina muestra una acción más intensa que la ouabaína sobre la velocidad de conducción aurículo ventricular, lo que se traduce en mayor efecto sobre el intervalo P-R del electrocardiograma.

Tercero: También es más pronunciado el efecto de la digitoxina sobre el período refractario del sistema de conducción aurículo-ventricular. Esto ha sido demostrado por dos métodos experimentales: uno, acción sobre el intervalo mínimo entre dos respuestas ventriculares transmitidas desde la aurícula estimulada con pares de choques que se acortan progresivamente; otro, estimulación de la aurícula a frecuencia creciente, hasta que el ventrículo no es capaz de seguir a todos los impulsos auriculares y se produce bloqueo aurículo-ventricular 2:1. Esta diferencia explicaría la superioridad de la digitoxina sobre la ouabaína para disminuir la frecuencia ventricular en la fibrilación auricular.

Variaciones en la eliminación urinaria de azur-A en el curso de la administración repetida oral del colorante incorporado a una resina de intercambio catiónico. G. ALCÁNTARA *, H. VIDRIO * Y E. PARDO C. * *(Departamento de Farmacología, Escuela Nacional de Medicina, U. N. A. M., México, D. F.).*

En el curso de un estudio del efecto de estimulantes e inhibidores de la secreción gástrica sobre la eliminación urinaria de azur-A por ratas que recibían el colorante incorporado a una resina de intercambio catiónico (Diagnex-Improved),

* Las personas cuyos nombres aparecen seguidos de un asterisco participan en el Congreso por invitación de alguno de los miembros de la Sociedad.

se hicieron 10 réplicas semanarias de un experimento en el que cada uno de los mismos cuarenta animales recibía una cantidad igual de resina por vía oral y era sometido, además, a cualquiera de 10 procedimientos experimentales (comida, inyección de insulina, de histamina, de colina, de carbacol, de cafeína, administración oral de alcohol, testigo, testigo, testigo). El tratamiento aplicado en cada ocasión y el orden seguido cada vez fueron determinados por métodos de azar, de manera que al final de las diez semanas cada animal terminó habiendo sido sometido una vez a cada "estímulo" y habiendo sido tres veces "testigo". Es la intención de este informe comunicar los cambios habidos en la eliminación del colorante en el curso de las diez semanas que duró el experimento. El análisis estadístico indicó que hubo un aumento progresivo significativo en la cantidad media de colorante eliminado por la población total de ratas, y que este aumento fué claramente superior cuando se consideraron aisladamente los "testigos" de cada réplica, de manera que, mientras que algunos de los "estímulos" produjeron aumento aparente en la eliminación del colorante (estímulo secretor) en las primeras semanas del experimento, en las semanas finales la eliminación fué mayor en los animales "testigo". La posibilidad peculiar de condicionamiento aparente merece estudios adicionales.

Interacción de los potenciales evocados en las áreas específicas visual y auditiva. M. ALCARAZ V. * Y C. GUZMÁN F. *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Recientemente se ha encontrado que la respuesta cortical a un estímulo significativo por condicionamiento, se modifica aumentando el componente negativo del potencial primario. Junto a estos cambios, las respuestas corticales evocadas por estímulos no significativos, se deprimen cuando son precedidas por el estímulo que ha adquirido significación [2]. Con apoyo en el hecho de que la estricnina aumenta el componente negativo de los potenciales evocados en la corteza visual [1], puede pensarse que empleando dicha droga es posible activar parcialmente el mecanismo que controla la transmisión aferente.

En el "encéfalo aislado" de gato, se estudió la interacción de las respuestas corticales a dos estímulos diferentes (visual y auditivo); se encontró que no existe modificación de dichas respuestas corticales ([3]). Cuando se aplicó estricnina en el área visual, se observó que al mismo tiempo que aumenta el componente negativo de la respuesta primaria al estímulo luminoso, existe depresión de la respuesta cortical evocada por un ruido, que se aplica 100 a 200 milisegundos después del estímulo luminoso. No se encontraron modificaciones importantes en los potenciales que por estímulos luminosos y auditivos, fueron evocados en la formación reticular.

Los resultados muestran que las modificaciones que imprime la estricnina al funcionamiento cortical, activan en forma parcial el mecanismo que interviene en el control de la transmisión aferente, estudiado en el proceso de condicionamiento ([2]). No es posible precisar, en estas condiciones experimentales, hasta qué punto interviene la formación reticular en dicho mecanismo.

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Difusión del azul tripán en membranas nictitantes denervadas. F. ALONSO DE FLORIDA * Y L. RAMÍREZ NÁJERA.* *(Departamento de Fisiología, Escuela Nacional de Medicina, U. N. A. M., México, D. F.).*

Aumento de la permeabilidad celular y de la permeabilidad capilar, así como disminución de la destrucción de los mediadores químicos, han recibido apoyo experi-

mentalmental como factores involucrados en la potenciación inespecífica a los agentes humorales en tejidos privados de su inervación. No han sido motivo de estudios anteriores los cambios que posiblemente pueden ocurrir en la velocidad de difusión de las substancias estimulantes consecutivamente a la denervación.

Esta nota da cuenta de algunos experimentos preliminares encaminados al análisis de tal posibilidad. Se consideró adecuado estudiar, como un primer paso, la difusión de substancias de moléculas relativamente grandes carentes en sí mismas de acción estimulante sobre el tejido.

A 20 gatos se les extirpó uno de los ganglios simpáticos superiores y transcurridos de 14 a 17 días, se les inyectó intramuscularmente, en ambas membranas nictitantes, bajo anestesia con nembutal, 0,0125 ml de una solución de azul tripán (5 mg ml). Dos horas después las membranas les fueron extirpadas, fijándolas por sus bordes en unas tablitas de modo que conservaran su forma y dimensiones, a continuación fueron puestas en un desecador durante tres o cuatro días. Se midieron entonces en cada una de ellas los diámetros mayor y menor de la mancha azul de forma aproximadamente elíptica. Finalmente fué calculada la diferencia entre las áreas correspondientes al lado intacto y al lado denervado. Las diferencias encontradas dieron para el grupo de observaciones un incremento de 13.34 ± 4.3 (error standard) a favor del lado denervado, que corresponde a un aumento del 26.7 %. El valor de la probabilidad, P. 0.01, se calculó por medio de rangos. Tales resultados apoyan la hipótesis de que la difusión acelerada es factor digno de considerarse en la sensibilización consecutiva a la denervación.

Intervención del sistema nervioso en la regulación de la glicemia. R. ALVAREZ-BUYLLA, J. CARRASCO-ZANINI * Y E. R. DE ALVAREZ-BUYLLA *. *(Departamento de Fisiología, Escuela Nacional de Ciencias Biológicas, I. P. N., México, D. F.).*

Si durante ocho días seguidos se inyecta insulina a un perro, haciendo coincidir su efecto hiploglicemiante con el sonido de un metrónomo o de un timbre, al noveno día, el sonido del metrónomo o del timbre producen un efecto hipoglicemiante análogo al que habían producido las inyecciones de insulina.

Se consiguió obtener hipoglicemias condicionales semejantes en perros con diabetes aloxánica y en perros con diabetes por extirpación total del páncreas. Tales hechos nos hicieron pensar que en los descensos del azúcar sanguíneo que producen los estímulos condicionales, no interviene la insulina.

Como estos hechos son incompatibles con la teoría actual acerca del mecanismo de acción de la insulina, se pensó que hay un mecanismo reflejo que interviene en la regulación de la glicemia y que la hipófisis podría ser el efector humoral del mismo.

En perros hipofisoprivos se demostró que no es posible establecer el reflejo condicional hipoglicemiante, a pesar de que en estos perros, se establecen otros reflejos condicionales con características semejantes a perros normales.

En la actualidad se hacen experimentos encaminados al estudio del componente aferente y central de este mecanismo reflejo que interviene en la regulación de la glicemia.

Potenciación adrenérgica de los efectos pupiloconstrictores del nervio motor ocular común. G. ANGUIANO L., A. VILLASANA E. * Y R. R. CHAVIRA *. *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Se sabe que las respuestas de algunos efectores a los impulsos colinérgicos, se potencian después de estimular su inervación adrenérgica, o de inyectar adrenalina o noradrenalina. En este trabajo se comunica el efecto potenciante de la estimulación

del simpático sobre las respuestas del iris a los impulsos de los nervios ciliares. Este efecto se presenta de un modo óptimo, veinte a treinta segundos después de estimular el simpático; la potenciación perdura por algunos minutos; y es más notoria cuando antes del experimento se inyecta una dosis única de cinco a diez microgramos de prostigmina. Dentro de ciertos límites, mientras más se estimula al simpático, mayor es la respuesta del músculo constrictor de la pupila a la estimulación parasimpática. Cuando se están estimulando los nervios ciliares y se agrega la estimulación del simpático, el efecto de ésta, no hace crecer el perímetro pupilar; en cambio, se observa que la pupila empieza a disminuir de diámetro, acentuándose la constricción después de que cesa el estímulo simpático.

La adrenalina, noradrenalina, adrenocromo y 5-hidroxitriptamina producen los mismos efectos que la estimulación del simpático.

Los autores se inclinan a pensar que el efecto que se describe se debe, entre otras causas, a que los mediadores del simpático se convierten en compuestos indólicos, los cuales poseen actividad anticolinesterásica.

La estimulación del parasimpático no influye sobre las respuestas pupilares simpáticas.

Efecto del veneno de alacrán sobre la actividad eléctrica cortical. C. BEYER F. * Y E. AGUADO S. *. *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Sampayo en 1944 ([3]), señaló que el veneno de alacrán produce bloqueo de las ondas lentas del electroencefalograma, concluyendo que actúa sobre la corteza cerebral. Trabajos realizados en este laboratorio han demostrado que la aplicación local de veneno de alacrán a la corteza cerebral, no tiene ningún efecto, y si éste se presenta es tardío y generalizado, posiblemente por difusión en el líquido céfalo-raquídeo y no por acción directa. Barros da Fonseca ([1]) demuestra, en estudios histológicos, que la toxina de alacrán lesiona los núcleos autónomos del tallo cerebral. Moruzzi y col. ([2]) han localizado en la substancia reticular del tallo cerebral y en el hipotálamo un sistema facilitador ascendente que desincroniza la actividad eléctrica cortical. Estos hechos sirvieron de base para tratar de aclarar el mecanismo que interviene en la desincronización cortical producida por la toxina escorpiónica.

Por la inyección de pequeñas dosis de cloralosa, o bien por lesión de la formación reticular mesencefálica, se obtuvieron ondas lentas en el registro eléctrico de la corteza cerebral de gatos. En los animales anestesiados con cloralosa la estimulación de la substancia reticular, produjo desincronización. La aplicación de veneno de alacrán (dosis de 0.1 de glándula en 0.01 cc de suero), en la formación reticular mesencefálica, provocó de inmediato el mismo efecto que la estimulación eléctrica de la reticular. Tardíamente se observó un aplanamiento de la actividad eléctrica. En los gatos con lesión de la formación reticular mesencefálica, la aplicación de veneno en el hipotálamo posterior ocasionó aplanamiento de las ondas lentas.

Los resultados muestran que el veneno activa el sistema reticular facilitador ascendente, activación que se manifiesta por desincronización del electroencefalograma. Posteriormente tiene una acción de bloqueo sobre las neuronas corticales.

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Acción comparativa de agentes de bloqueo ganglionar sobre la transmisión en los sistemas simpático y parasimpático. J. CATO D. *, F. VELÉZ Y C. NÁJERA. *(Departamento de Farmacología, Escuela Nacional de Medicina, U. N. A. M., México, D. F.).*

Se ha comunicado (Pardo y col., *J. Pharm. exp. Therap.*, 1956, *116*, 376)

la selectividad aparente de la etil-metil-iso-octenil amina (EMOA) para el bloqueo de los ganglios del parasimpático. Como tal selectividad podría significar diferencias en la transmisión a través del simpático y el parasimpático, se hicieron experimentos en gatos para analizar la posible selectividad de otros agentes del bloqueo ganglionar carentes de acción atropínica. Se incluyeron en el estudio la propia EMOA, el tetraetilamonio, el hexametonio, la tubocurarina y la efedrina, esta última como representante de las aminas adrenérgicas, cuyos efectos ganglionares han sido analizados por Magaña y col. (Primer Congreso de la Sociedad Mexicana de Ciencias Fisiológicas, 1958). Se eligió el bloqueo de los efectos sobre frecuencia de la estimulación del vago como representativo de una acción sobre el parasimpático y el bloqueo del reflejo carotídeo como representativo de una acción sobre el simpático. Se hicieron experimentos suficientes para construir curvas de dosis respuesta para cada una de las substancias en relación a ambos efectos. Todas las substancias probadas mostraron selectividad para bloqueo del parasimpático del tipo descrito para la EMOA. Las diferencias cuantitativas no fueron significativas, si se consideran las objeciones que pueden merecer los parámetros experimentales como medidas cuantitativas del bloqueo en cada estructura. La selectividad común a los diversos agentes indica diferencias entre las estructuras del simpático y del parasimpático. Las del simpático pudieran ser menos sensibles que las del parasimpático o pudieran diferir de éstas en su irrigación, de manera que fuera distinto el curso temporal del equilibrio con el agente administrado por vía intravenosa. También es posible que el fenómeno pudiera resultar de la existencia de fibras sin relevo ganglionar del tipo sugerido inicialmente por Moe. (Pardo y col., *Amer. J. Physiol.*, 1950, *161*, 245).

La influencia de la frecuencia y de la estimulación del vago sobre la propagación aurículo-ventricular (A-V) del corazón de la tortuga. E. DEUTSCH. *(Departamento de Fisiología, Instituto Nacional de Cardiología, México, D. F.).*

Los aumentos de frecuencia cardíaca acortan la duración del electrograma auricular y a partir de determinada frecuencia de estimulación auricular, alargan la latencia A-V. Al controlar a frecuencia constante el corazón por medio de estímulos (S_1) e intercalando el choque adicional de prueba (S_2), se observa que la disminución del intervalo S_1-S_2 tiene el mismo efecto sobre la pausa A-V y el electrograma auricular que los aumentos de frecuencia.

La estimulación del vago a una frecuencia de 5/seg acorta el electrograma auricular y la latencia A-V, y disminuye la frecuencia cardíaca. Con la misma frecuencia de estimulación vagal y con el corazón controlado a frecuencia constante, el intervalo A-V se alarga y el acortamiento del fenómeno eléctrico auricular es similar al anterior. Cuando la estimulación del vago se hace a una frecuencia menor de 2/seg, los efectos sobre el ritmo del corazón y el tiempo de activación A-V son mínimos y también se acorta el potencial de acción auricular.

La determinación del período refractario funcional (PRF) de la aurícula, de la transmisión A-V y del ventrículo, informa que la aurícula es la que posee el PRF más corto y la propagación A-V el más largo. Al aumentar la frecuencia cardíaca el PRF del ventrículo y de la transmisión A-V se acortan. Dicho acortamiento es mayor para el ventrículo que para la activación A-V.

Se concluye en el presente trabajo que la teoría sugerida por Gilson de que la estimulación del ventrículo sucede al final del potencial de acción auricular y está en relación directa con la duración de éste, es inadecuada para explicar las presentes observaciones. Se presenta la evidencia experimental que demuestra la existencia de una estructura intermediaria entre las aurículas y el ventrículo, cuyas propiedades fisiológicas explican el retardo en la transmisión aurículo-ventricular.

Efecto de la estimulación eléctrica cortical sobre unidades aisladas del mesencéfalo. A. FERNÁNDEZ G.* Y E. ROLDÁN R.* *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Se ha encontrado que existen diferentes tipos de unidades aisladas mesencefálicas. Un 60 % de éstas no responde a estímulos aferentes. El resto muestra cambios de frecuencia por estimulación cortical o periférica.

Los potenciales evocados recogidos con macroelectrodos en la formación reticular mesencefálica se encuentran deprimidos en el período post-convulsivo, mientras que la actividad registrada es rápida y de bajo voltaje. Mediante el empleo de microelectrodos se observa que en este período, tras la convulsión inducida con metrazol, existen diferentes tipos de respuesta en las unidades aisladas reticulares, siendo el más común la aceleración de la frecuencia de descarga.

Con el objeto de evitar la acción masiva del metrazol se inició este estudio estimulando con corriente eléctrica la corteza cerebral, en gatos sin anestesia y curarizados. El lugar elegido fué la circunvolución suprasilviana anterior. Se registró corticograma en la región homóloga del hemisferio opuesto y unidades aisladas del mesencéfalo mediante microelectrodos orientados estereotáxicamente.

La corteza cerebral fué estimulada con un carrete de inducción, aplicando choques simples y faradicos convulsionantes.

Se encontró que la estimulación de las áreas de asociación corticales provoca una aceleración de la frecuencia en las unidades "espontáneas" y por el contrario, depresión duradera de las unidades mesencefálicas que intervienen en la transmisión aferente.

Los resultados sugieren la existencia de unidades reticulares que pertenecen a sistemas funcionales diferentes y con un distinto tipo de respuesta ante la activación aferente o cortical.

Las descargas repetidas en la aurícula de los mamíferos producidas por la estimulación del vago. J. GARCÍA RAMOS. *(Departamento de Fisiología, Escuela Nacional de Medicina, U. N. A. M., México, D. F.).*

Durante, o inmediatamente después de la estimulación del vago, pueden aparecer descargas repetidas de impulsos que son de origen auricular. El número de estas descargas varía desde una sola extrasístole, que ocurre con un intervalo constante después del impulso normal anterior, hasta un número indefinido que, con frecuencia relativamente alta, persisten durante todo el tiempo que se mantenga al vago estimulado.

El intervalo entre la extrasístole y el impulso normal que la precede fué prácticamente el mismo en las diversas observaciones realizadas, así como en los diversos ejemplos que se hallan en la literatura. Estos impulsos extrasistólicos son originados en el músculo auricular mismo.

Los datos encontrados sugieren que el mecanismo de su producción es a través de la estimulación del tejido por la diferencia de potencial existente entre dos regiones vecinas desigualmente afectadas por la acetilcolina liberada durante la actividad vagal.

Se demuestra la existencia de tal diferencia de potencial, que se exagera durante el desarrollo del componente lento del electrograma auricular y que tiene las mismas relaciones temporales de éste. Es de magnitud suficiente como estímulo, dada su intensidad, su duración y la constante de espacio del electrotono.

Las variaciones de esta diferencia de potencial como estímulo, y las de la excitabilidad del tejido vecino bajo su influencia, hacen críticas las condiciones para que se observen las descargas repetidas. Si experimentalmente se cambian tales factores en forma apropiada, estas descargas se pueden presentar con mayor facilidad.

La formación reticular como un mecanismo de control de la transmisión aferente. C. GUZMÁN F. Y M. ALCARAZ V. * *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Se ha demostrado que la interacción de las respuestas a estímulos aferentes, en sus respectivas áreas de proyección, sólo existe cuando la corteza cerebral se activa con estricnina (1). En un trabajo no publicado, fué difícil reproducir la acción supresora de la formación reticular sobre las vías visuales (3), al estimular eléctricamente dicho sistema. En el presente estudio se intentó dilucidar si por medio de la estricnina era posible activar el mecanismo supresor localizado en la formación reticular.

En el "encéfalo aislado" de gato, se colocaron electrodos en la formación reticular (plano A2, L2, H-3) y en la corteza auditiva. Los potenciales se evocaron empleando estímulos luminosos de corta duración, los cuales precedían 200 milisegundos a los estímulos auditivos. La estricnina se aplicó en la formación reticular por medio de una pequeña cánula, que se orientó con el aparato estereotáxico en el plano 0, L2, H-2. La estricnina se usó a la concentración de 0.2 %; la cantidad inyectada fué de 0.01 de cc.

Los resultados muestran que la aplicación de estricnina en la formación reticular produce depresión de los potenciales evocados en la corteza auditiva, cuando van precedidos de estímulos luminosos.

De los resultados experimentales, se infiere que la formación reticular interviene en el mecanismo que controla la transmisión aferente.

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Efectos ganglionares de algunas drogas adrenérgicas. J. L. MAGAÑA *, J. UGALDE * Y E. PARDO C. * *(Departamento de Farmacología, Escuela Nacional de Medicina, U. N. A. M., México, D. F.).*

Se analizaron los efectos ganglionares de algunas aminas adrenérgicas como parte de un programa de exploración de dichos efectos en derivados secundarios y terciarios de nitrógeno. La efedrina, la amfetamina, la fenilefrina, el isopropilarterenol y la metil-iso-octenil-amina se administraron a gatos, en infusión continua, a velocidad constante, y se observó la acción sobre la respuesta a la estimulación eléctrica del vago periférico, sobre la respuesta a la oclusión de las carótidas, sobre las respuestas presoras de la epinefrina y sobre las respuestas hipotensoras y presoras de la acetilcolina. En general, las diversas aminas probadas interrumpieron el efecto sobre frecuencia cardíaca de la estimulación eléctrica del vago sin antagonizar los efectos de la acetilcolina sobre los efectores periféricos. En ocasiones parecieron potenciar estos efectos periféricos, posiblemente como resultado del bloqueo de las acciones ganglionares de la propia acetilcolina. Tal antagonismo a nivel ganglionar pudo mostrarse en el caso de todas las substancias probadas, e incluso, fué posible en ocasiones, invertir los efectos de la acetilcolina en el animal eserinizado y atropinizado. En general, la interrupción del reflejo carotídeo fué mucho menos completa que la de la transmisión vagal, mostrando las diversas aminas adrenérgicas selectividad para el parasimpático del tipo que comunican Cato y col. (Primer Congreso de la Sociedad Mexicana de Ciencias Fisiológicas, 1958), para otros agentes del bloqueo ganglionar. Ocasionalmente se observó potenciación del reflejo carotídeo, posiblemente resultado de la sensibilización a los efectos periféricos de los mediadores adrenérgicos. El análisis cuantitativo de los resultados indica el siguiente orden de actividad sobre la transmisión vagal: isopropilarterenol > efedrina > amfetamina > metil-iso-octenil-amina > fenilefrina. El número de substancias probadas fué demasiado pequeño

para permitir conclusiones válidas acerca de las relaciones entre la estructura química y la actividad.

Los períodos refractarios absoluto y efectivo de Lewis. C. MÉNDEZ. *(Departamento de Farmacología, Instituto Nacional de Cardiología, México, D. F.).*

La escuela de Lewis considera que el verdadero período refractario absoluto corresponde al intervalo más corto entre dos estímulos que, aun cuando el segundo no origine una respuesta propagada a distancia, pospone o previene la respuesta provocada por un tercer estímulo que sigue al segundo rápidamente. El segundo estímulo origina, según Lewis, una respuesta que únicamente se propaga una pequeña distancia. Según esto, el período refractario absoluto clásico no señalaría el momento en que el músculo comienza a ser excitable, sino aquel que el impulso podría transmitirse a distancia. Por este motivo la denominaron período refractario efectivo.

Como no existe evidencia en favor de que el segundo estímulo origine un potencial de acción, hemos estudiado las modificaciones que produce dicho segundo estímulo sobre la curva de recuperación de la excitabilidad en el corazón de mamíferos con estos resultados:

Primero: Si el choque es catódico, inmediatamente después de aplicado y durante unos milisegundos se obtiene facilitación seguida de una fase de depresión. Durante ésta, el segundo choque puede abolir la respuesta a un tercer estímulo que siga al segundo rápidamente.

Segundo: Si se aumenta la intensidad del segundo choque, la facilitación y la depresión obtenidas son de mayor magnitud. El fenómeno es gradual: no sigue la ley del todo o nada.

Tercero: Invirtiendo la polaridad del choque se obtiene primero, depresión seguida por facilitación.

Cuarto: La quinidina y los digitálicos, que según Lewis acortan el verdadero período refractario absoluto y alargan el efectivo, únicamente acentúan las modificaciones ya señaladas.

De estos resultados se llega a la conclusión de que el segundo choque únicamente da lugar a cambios de excitabilidad del tejido análogos a los obtenidos en tejidos completamente recuperados empleando choques subumbrales; es decir, el verdadero período refractario absoluto de Lewis es un artefacto experimental.

Efecto de la veratramina sobre las acciones cardíacas de la adrenalina. R. MÉNDEZ, C. MÉNDEZ Y A. MORALES *. *(Departamento de Farmacología, Instituto Nacional de Cardiología, México, D. F.).*

Krayer y colaboradores han demostrado en los últimos años que el efecto cronotrópico de la adrenalina puede ser antagonizado mediante la administración de veratramina, alcaloide amínico secundario de *Veratrum viride* y *V. album*. Este antagonismo se deja sentir en la acción cronotrópica de la adrenalina sobre el nodo seno auricular y sobre el aurículo ventricular. Sin embargo, la veratramina no antagoniza el efecto inotrópico positivo de la adrenalina ni su acción sobre el período refractario de la conducción aurículo ventricular.

Nuestros experimentos demuestran que la veratramina por sí sola produce efecto cronotrópico negativo, alarga los períodos refractarios auricular y ventricular y no modifica la excitabilidad ni la conducción de la aurícula y del ventrículo.

La veratramina no es capaz de antagonizar ni aun en dosis elevadas los efectos de la adrenalina sobre excitabilidad, velocidad de conducción y período refractario de la aurícula y del ventrículo. Tampoco antagoniza el automatismo de la aurícula, llamado actividad autosostenida lenta, que se despierta en la orejuela por estimulación eléctrica bajo la acción de la adrenalina.

La veratramina antagoniza, pues, selectivamente la acción de la adrenalina sobre los automatismos seno auricular y aurículo ventricular, no antagonizando la actividad ectópica señalada anteriormente. Tampoco actúa sobre los efectos de la adrenalina en otras propiedades fisiológicas del corazón.

Efectos de la isquemia y perfusión sobre la actividad del ganglio simpático. J. NEGRETE M. Y G. YANKELEVICH N.* *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Se estudió la actividad del ganglio simpático cervical superior en gatos anestesiados con nembutal. Los potenciales de acción recogidos en las fibras postganglionares o las contracciones de la membrana nictitante, se tomaron como índice de dicha actividad.

La frecuencia mínima de estimulación de fibras preganglionares que produjo fatiga de transmisión, en experimentos con registro eléctrico de postganglionares, fue de 15 por segundo. En 30 experimentos en los que se practicó el registro de la contracción de la membrana nictitante, durante la estimulación de las fibras preganglionares a distintas frecuencias, se encontró que 15 minutos de isquemia produjeron cambios en el mantenimiento de la tensión, que pueden ser clasificados en 5 grupos, a saber:

a) Un aumento tensional seguido de retorno al nivel previo por supresión de la isquemia.

b) Un aumento tensional precedido de una caída. El de pinzamiento es seguido de un aumento adicional y transitorio de la tensión.

c) La isquemia produce caída tensional y retorno de ésta a su nivel inicial al restituir la circulación.

d) Una caída tensional como en *c*, con un pequeño aumento transitorio intercalado.

e) Sin cambio.

Ninguno de estos grupos se puede obtener en las mismas condiciones durante la estimulación de postganglionares, excepto *e*.

Los tipos *a*, *b*, *c* y *d*, se producen a frecuencias altas (15 a 60 por segundo). A frecuencias bajas (2 a 5 por segundo), generalmente no hay cambios tensionales.

Los efectos de tipo *b* y *d* se pueden considerar como intermedios de los tipos fundamentales *a* y *c*.

El aumento de tensión de tipo *a*, se transforma en *c* por la repetición de la isquemia.

Los cambios tensionales descritos pueden ser el resultado de la suma algebraica de un aumento de excitabilidad a la acetilcolina de las células ganglionares más una disminución de la liberación del transmisor.

La perfusión del ganglio con Locke fosfato y Locke carbonato oxigenados, reproduce los efectos de isquemia descritos.

Tanto la isquemia como la perfusión con Ringer oxigenado, son capaces de producir actividad de las células de los ganglios no estimulados. Solamente la perfusión con sangre desfibrinada no altera la actividad ganglionar en reposo o durante la estimulación a frecuencias fatigantes.

Nueva valoración del efecto de la estrictina sobre la susceptibilidad del ratón a la poliomielitis experimental. E. PARDO C.*° Y K. W. COCHRAN*°°. (° *Departamento de Farmacología, Escuela Nacional de Medicina, U. N. A. M., México, D. F.* - °° *Escuela de Epidemiología, Universidad de Michigan).*

La sugestión de que la actividad en las neuronas motoras pueda modificar la

susceptibilidad de éstas al virus de la poliomielitis (1), llevó al análisis de los efectos de drogas depresoras y estimulantes del sistema nervioso central sobre la poliomielitis experimental en el ratón. En un trabajo previo (2), se comunicó que la estricnina aceleró la muerte de ratones inoculados por vía intraespinal con virus de Tipo I, y aumentó aparentemente el título mismo del virus. Como tal aumento sería difícil de explicar a la luz de las teorías de partícula infectante comúnmente aceptadas, y como se encontró en el mismo trabajo que la estricnina producía mortandad apreciable en animales que recibieron inyecciones intraespinales de médula de ratón sin virus, se hicieron experimentos en que se analizó el efecto de la adición de estricnina a la dieta normal (0,25 mg./g. de alimento) sobre la rapidez de aparición de parálisis en grupos de ratones inoculados por vía intraperitoneal con una cepa MEF_1, adaptada a esta vía. Se estudió además la susceptibilidad residual a la poliomielitis de la población de ratones que sobrevivieron a estos experimentos. En otros animales se midió la influencia de la estricnina sobre la rapidez de multiplicación del virus en el sistema nervioso central. Los resultados permitieron: (a) confirmar el efecto de la estricnina sobre la rapidez de aparición de la parálisis; (b) concluir que el aumento de título previamente comunicado debía atribuirse a la potencialidad de la toxicidad de la estricnina por la inyección intraespinal de material nervioso, ya que no fué aparente cuando se utilizó la vía intraperitoneal de inoculación; y (c) que la estricnina no produjo infecciones subletales que dieran inmunidad activa a los animales tratados. Los estudios hechos no permitieron hacer conclusiones acerca del efecto de la droga sobre la curva del crecimiento del virus en animales individuales.

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Estudio de algunas características del sistema vascular en la preparación de pulmón aislado del conejo. R. REYNAUD ACOSTA * ° Y J. GARCÍA RAMOS °°. (°*Departamento de Fisiología, Instituto Nacional de Neumología,* - °° *Departamento de Fisiología, Escuela Nacional de Medicina).*

En la preparación de pulmón aislado del conejo, perfundida con solución salina, se determinaron el gasto circulatorio total y el gasto de líquido que, después de extravasarse, pasa a los alvéolos pulmonares y escurre luego por las vías aéreas.

Para presión de perfusión constante, la cantidad de líquido que escurre por la tráquea es proporcional a la magnitud de la superficie a través de la cual se hace la filtración o extravasación. En estas condiciones, las variaciones en esta cantidad indicarán variaciones paralelas en el número de capilares alveolares activos. Se obtienen tales variaciones por la adición de substancias que tienen acción vasomotora, como la adrenalina.

En determinadas circunstancias, es posible obtener cambios en el número de los capilares activos, sin modificaciones paralelas en el gasto circulatorio total. Esto sugiere la existencia de una distribución especial de los mecanismos de regulación vasomotora de este sistema vascular.

Irradiación de los potenciales visuales evocados a las áreas de asociación. E. ROLDÁN R. * Y A. FERNÁNDEZ G. * *(Departamento de Fisiología, Instituto de Estudios Médicos y Biológicos, U. N. A. M., México, D. F.).*

Durante el proceso de condicionamiento a un estímulo luminoso, los potenciales evocados, originalmente limitados a las áreas de proyección específica, aparecen en la porción anterior de la circunvolución suprasilviana (área A_3) (2 y 3).

Al colocar estricnina en el área visual de una preparación "encéfalo aislado" de gato se reproducen algunos de los fenómenos eléctricos que ocurren en el condicionamiento (1). Se sabe que en los animales no condicionados, los potenciales visua-

les evocados en el área A_3 son de pequeña magnitud o no se presentan; en este trabajo, se estudian las modificaciones de las respuestas evocadas en el área A_3, producidas por la estricnización del área visual.

En gatos curarizados y sin anestesia se expuso la corteza cerebral de un hemisferio y se colocaron ocho electrodos superficiales sobre las siguientes áreas: visuales I y II, A_3 y sensitivo motora. Se registró con un electroencefalógrafo Grass de ocho canales. El estímulo luminoso se aplicó mediante un *flash* electrónico con ráfagas de 1|250 de segundo de duración.

Al aplicar estricnina en el área visual a la concentración de 0.5% ó 1%, los potenciales evocados en dicha región aumentan de amplitud; al mismo tiempo aparecen potenciales en el área A_3, que crecen con el tiempo.

Los resultados sugieren que el mecanismo cortical, activado por la estricnina, interviene en la producción de los signos eléctricos que acompañan al condicionamiento.

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Las constantes dinámicas de Hill en las contracciones isotónicas. A. ROSENBLUETH Y R. RUBIO *. *(Departamento de Fisiología, Instituto Nacional de Cardiología, México, D. F.).*

Hill (1938) sugirió que la velocidad de acortamiento (v) de un músculo estriado varía con la carga (P) según la ley $(P + a)(v + b) = b(P_0 + a)$, donde a, b y P_0 son constantes.

De hecho, la velocidad no depende tan sólo de la carga. Es función del tiempo, de la frecuencia de estimulación, de la longitud inicial y de la longitud a la cual se mide. Varía cuando se cambia la longitud inicial, manteniendo constante la carga y la longitud a la cual recibe el músculo dicha carga.

La ley de Hill es una relación empírica aplicable sólo a las contracciones tetánicas con carga tardía y longitud inicial fija. Aún entonces, los parámetros a, b y P_0 no son constantes sino que requieren ajuste para cada longitud y frecuencia de estimulación. La sugestión de Hill de que estos parámetros son constantes características para cada músculo es así inaceptable.

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Las contracciones isotónicas de los músculos estriados. R. RUBIO G. * *(Departamento de Fisioterapia, Hospital Infantil, México, D. F.).*

Tanto el músculo como la fibra muscular estriada son sistemas elásticos heterogéneos. Incluyen elementos elásticos pasivos en paralelo y en serie con los elementos contráctiles. Esta estructura hace imposible obtener contracciones isotónicas o isométricas puras.

Se empleó el gastrocnemio de la rata. En unos experimentos se registró independientemente de cada uno de los gemelos aplicando estímulos máximos al poplíteo con frecuencias de 50 a 70 por segundo. Los diagramas de tensión longitud (T-L) de las dos fracciones se superponen al ajustar las escalas de los ejes de las tensiones de tal modo que guarden la misma razón que las dos masas musculares.

En otros experimentos se colocaron dos pares de electrodos de estimulación en el poplíteo y se hizo una sección parcial del nervio entre ellos. El par inferior activaba todas las fibras motoras, el superior sólo una fracción. Los diagramas T-L no se pueden ajustar como antes. Conociendo el diagrama T-L de reposo de las fibras

que no se contraen, se puede corregir el correspondiente a la fracción de fibras activas y obtener el diagrama real de éstas.

Estos resultados indican que al acortarse un músculo cambia la distribución de la carga entre los elementos activos y los elásticos pasivos en paralelo. Como consecuencia, la contracción no es isotónica.

En la contracción llamada isométrica, como señaló Hill, los elementos activos estiran a los elásticos pasivos en serie, y el acortamiento de los activos impide que la contracción sea propiamente isométrica.

Efectos acumulativos producidos por la anoxia sobre algunas propiedades de los tejidos cardíacos y del sistema vascular. P. RUDOMIN Z. * Y E. DEUTSCH *. *(Departamento de Fisiología, Instituto Nacional de Cardiología, México, D. F.).*

Los experimentos fueron realizados en perros anestesiados y con el tórax abierto. La anoxia se produjo haciendo respirar al animal una mezcla de oxígeno al 8% en nitrógeno.

La anoxia produce en el animal íntegro una elevación temporal de la presión arterial seguida de un descenso que se mantiene durante la anoxia.

Al producir la anoxia durante períodos sucesivos de diez minutos, intercalando períodos de reposo de la misma duración, la elevación de la presión arterial es cada vez menor y alcanza su máximo con una latencia más corta y con mayor rapidez. La velocidad de caída también se va acentuando. Durante el tiempo que dura la anoxia se observa una notable disminución refleja de la frecuencia cardíaca. En las series sucesivas esta disminución es cada vez menor.

En la mayoría de los casos, la latencia aurículo ventricular se alarga durante la fase inicial de la anoxia. Al prolongarse la misma puede presentarse también un acortamiento. En ocasiones sólo se observa este acortamiento.

Cuando la presión arterial cae a valores menores de 30 mm. de Hg. aproximadamente, la frecuencia cardíaca disminuye y la latencia aurículo ventricular se alarga hasta que aparece el bloqueo aurículo ventricular.

La velocidad de conducción auricular no sufre cambios durante las series de anoxia; sólo cuando la presión arterial alcanza valores de 30 mm. de Hg. o más bajos, ésta empieza a disminuir notablemente.

La anoxia produce primero un aumento de la excitabilidad eléctrica del músculo auricular y ventricular. Al prolongarse la anoxia el umbral sube gradualmente, aun cuando se interpongan períodos de reposo.

La magnitud del potencial de acción auricular, monofásico, cae durante la anoxia, recuperándola nuevamente en condiciones de reposo.

Las denervaciones parciales o totales del corazón disminuyen notablemente la magnitud de la respuesta presora y los cambios de frecuencia cardíaca provocados por la anoxia.

Efecto del di-isopropil-fluorofosfato sobre la secreción gástrica en el perro. E. TÉLLEZ GIRÓN *. *(Departamento de Ciencias Funcionales, Facultad de Medicina, Universidad Autónoma de San Luis, Potosí).*

Basados en los resultados obtenidos por diferentes autores, del efecto de inhibidores reversibles de la colinesterasa sobre la secreción gástrica (eserina, cafeína, bases cuaternarias de amonio, etc.), se planteó estudiar el efecto de otro compuesto de acción más específica y de carácter no reversible, el di-isopropilfluorofosfato (DPF), sobre la secreción gástrica de perros tanto basal como estimulada previamente con

histamina, así como su acción combinada con atropina, estudiando variaciones en volumen, acidez y actividad péptica.

Para el efecto, se inyectaron por vía intravenosa 20 mg. de DPF a perros con fístula gástrica.

En cada caso, después de la administración de la droga, se obtuvo respuesta secretoria gástrica. El efecto estimulante del DPF fue inhibido por un miligramo de sulfato de atropina inyectado por vía endovenosa una hora antes del DPF.

El DPF incrementó la respuesta secretoria gástrica cuando se estimuló ésta constantemente con histamina.

Se concluye que:

1) El DPF estimula la secreción gástrica por inhibición de la colinesterasa ocasionando menor destrucción de acetilcolina.

2) La acetilcolina es un importante mediador en las funciones secretorias del estómago.

3) El DPF a la dosis empleada incrementa la respuesta gástrica, estimulada con histamina. No se puede decir si se trata de un efecto aditivo o sinérgico.

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Effect of phosphorylation dissociating agents on carbon dioxide filation by bakers' yeast. by A. O. M. STOPPANI AND SUSANA L. S. DE FAVELUKES. *(Departamento de Bioquímica, Escuela de Medicina, Universidad de Buenos Aires).*

Azide and 2,4-dinitrophenol inhibit carbon dioxide fixation by baker's yeast. The anaerobic fixation is far less affected by 2,4-dinitrophenol than the aerobic one, which is attributed to the partial formation of the carbon dioxide acceptor (phosphoenol-pyruvate) by a non-oxidate mechanism (anaerobic glycolysis). When fermentation is not in operation, inhibitors of respiration (anti-mycine *A*) diminish carbon dioxide fixation. A direct action of 2,4-dinitrophenol and azide on the fixation reaction cannot be ruled out.

Action of 3-5-3' triyodothyronine on the kidney and renal hypertension in the rat. by J. A. ZADUNAISKY. *(Instituto de Fisiología, Facultad de Medicina, Buenos Aires).*

The effects of 3-5-3' triyodothyronine on the kidneys and blood pressure were studied in rats.

The results show that this hormone produces an important rise in the weight of the kidneys in normal rats, that completely disappears 20 days after discontinuing the treatment.

It also rises the lower renal weight of hypothyroid rats and rapidly stimulates the renal hypertrophy during the compensatory hypertrophy of the kidney of normals.

The lower values for insulin clearance observed in hypothyroid rats may also be restored to normal levels by the injection of the hormone.

Its administration to normal rats does not modify the blood pressure, but an important increase to hypertensive levels is produced when it is injected to rats with severe renal reduction (ligature in 8 of the left kidney and nephrectomy of the right). This last effect disappears when the treatment is stopped.

Effect of hypoglycemia due to insulin and tolbutamide on the testicle of the rat. by R. E. MANCINI, J. C. PENHOS, H. M. GERSCHENFELD AND I. IZQUIERDO. *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas e Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

Histological and histochemical studies of the effects of acute hypoglycemia provoked by insulin or tolbutamide on the testicle of inmature and adult rats were

performed. Used doses were: insulin, 5 to 40 u/100 g; Tolbutamide, 200 mg to 3 g/k. Three groups of rats were used as controls: a) Non-treated inmature and adult rats, b) receiving insulin or tolbutamide in addition to glucose (for preventing hypoglycemia), and c) glucose alone.

It was observed: 1) in inmature rats, congestion and slight edema in the intertubular space without alteration of the germinal epithelium; 2) in adult rats, in addition to congestion and edema, vacuolization of Sertoli cells and spermatogonia, and release of spermatocytes I and spermatids. On the other hand, no alterations in the Leydig cells or in the intertubular fibroblasts were observed; 3) these lesions were more frequent and more intense in adult rats than in younger animals; 4) vacuolization seems to be due to a hydropic imbibition process since histochemically, the reactions for lipids, glucolipids, glycogen and mucopolysaccharides are negative. PAS method stains a granular substance of unknown nature appearing in the Sertoli cells and spermatogonia; 5) the aforementioned lesions were more frequent and more intense during hypoglycemic coma, less frequent and slight pre-coma; 6) prevention of hypoglycemia by glucose administration inhibited development of these lesions which were only present in a significantly lower percentage of cases, while glucose given alone did not produce lesions in the testis; 7) lesions of chronic inflamatory type, completely different from those described during hypoglycemia was observed, in a certain number of controls.

Seasonal variation of the antidiuretic action of the encephalon and hypophysis of the toad. by JULIA URANGA. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires).*

The antidiuretic activity of different parts of the toad's brain has been studied.

1) The olfactory lobes, cerebral hemispheres, optic lobes, cerebellum, choroid plexus of the fourth ventriele, bulb and medulla, were found to be inactive both in summer and in winter.

2) Antidiuretic activity was found in the choroid plexus of the third ventricle, preoptic nucleus, infundibulum and neurohypophysis (neural intermediate lobe).

3) The antidiuretic activity of the neurohypophysis of the toad has seasonal variations: it is much higher in summer than in winter.

4) No sexual difference was found nor a decrease in neuro-hypophyseal activity due to different agents (light, darkness, heat or cold) or certain drugs (acetylcholine and adrenaline).

5) A slow dehydration produces a decrease of antidiuretic activity in the neurohypophysis, but if the dehydration is sharp, in three hours, there is no decrease of antidiuretic activity.

December 12th, 1957

Effect of some inhibitors of oxydative phosphorilation on carbon dioxide fixation by *S. Cerevisiae.* by A. O. M. STOPPANI AND SUSANA L. S. DE FAVELUKES. *(Cátedra Biológica, Facultad de Ciencias Médicas de Buenos Aires, y Laboratorio de Metabolismo Celular, Comisión Nacional de la Energía Atómica).*

Histochemical study of acid phosphatase in enchondral ossification. by FRITZ SCHAJOWICZ AND R. L. CABRINI. *(Laboratorio de Anatomía Patológica - Hospital Ramos Mejía, Buenos Aires).*

The histochemical distribution of acid phosphatase in enchondral ossification of mice, rats and man is described.

The maximum enzymatic activity is found in osteoclasts, chondroclasts and erosive zones of the ossifying vessels.

The growing cartilage reacts especially in the proliferating zones. There is a loss of enzymatic activity in the hypertrophic and calcified cartilage.

The possible role of acid phosphatase in the reabsorption process of bone cartilage is discussed.

Action of testosterone on S^{35} uptake by mucopolysaccharides of connective in the cock's comb. by R. E. MANCINI, I. IZQUIERDO AND P. KIRSCHBAUM. *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires, Argentina).*

The action of testosterone upon the comb of prepuberal cockerels and castrated adult cocks was studied histologically, histochemically and radioautographically. At the end of the treatment 2 uC per g of body weight of S^{35}-sodium sulphate was given intraperitoneally.

Combs were removed at periodic intervals of 2 hours until reaching 24 hours; they were placed in Bouin's fixative and 10 % formalin. The stripping-film radioautographic method was used and variations in their density were measured with a microscopic densitometric method.

It was observed: 1) in prepuber cockerels, local application of testosterone provoked an increase in comb's volume hyperplasia of young fibroblast with cytoplasmic basophilia and a dissociation of the collagen net with appearance of an interfibrillar mucopolysaccharid and intense incorporation of S^{35}. 2) In adult castrated combs fibroblasts diminished in number, the collagen net was reconstituted, mucopolysaccharides disappeared and S^{35} incorporation was mild. 3) In adult castrated cocks treated with testoterone, histological and histochemical modifications were similar to those of prepuberal cockerels to which testosterone was given, and S^{35} incorporation was intense. 4) Densitometric curves revealed a gradually increasing S^{35} incorporation from the first hours up to the 24th hour, both in adult normal and castrated cocks, and in prepuber cockerels treated with testosterone. 5) S^{35} localization was during the first hours chiefly pericellular, around the fibroblast, and, later, diffuse in the intercellular spaces. 6) Extraction of S^{35} from the slices was total with Barium hidroxide and partial with hyaluronidase. This suggests the existence of a sulphurated mucopolysaccharide in the cock's comb, which is susceptible of being regulated.

Synergic action of sulphonamides and insulin in recently pancreatectomized dogs. by E. J. URGOITI. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad de Buenos Aires).*

In order that the hypoglycemic sulfonamides may play their role, the presence of insulin is necessary, at small doses, insufficient by themselves to maintain a glycemic level.

Imbibition of tissues by insulin, prior to the administration of sulfonamides, is also necessary for their action to be exerted.

Mechanism of the pressor action of the spider "Latrodectus mactans" venom. by R. CALVO, I. J. CHIONETTI, J. C. FASCIOLO Y SEÑORES A. BINIA, M. M. PUEBLA, E. ZANGHERI AND F. FERNÁNDEZ. *(Departamento de Fisiología, Universidad Nacional de Cuyo, Mendoza, Argentina).*

To investigate the mechanism of the pressor effect produced by the venom

of the spider "Lactrodectus mactans", when injected intravenously, several organs were perfused by a modified Schuster-Dale Pump.

The intravenous injection of 1 ½ - 3 glands/kilo body weight caused an important increase in the arterial pressure of the dog and in the perfusion circuits of the leg, kidney and infradiaphragmatic organs, (this last perfused through the abdominal aorta), even when denervated surgically of by hexamethonium.

It is concluded that the pressor effect is due to an increase in the peripheral resistance, caused by an humoral agent.

Adrenalin and nor-adrenalin titration in Laewen-Trendelenburg preparation in the toad. by A. C. NACIMIENTO. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad de Buenos Aires).*

1) Some pharmacological properties of the Laewen-Trendelenburg preparation have been studied, with the following results: a) any assay of drugs by this method requires a large number of animals and measures of flow, an adequate planning of the experiments, a correct and indispensable statistical analysis. In short qualitative appreciations are not convenient. Besides, the measurements must be based on the precise mechanical registering of the number of drops. b) The possibility of a seasonal variation should be taken into account. c) The composition of the perfusion fluid may have a considerable influence when measuring the flow. d) The most convenient perfusion pressure in these experiments was 12 ± 1 cm of H^2O, that gives an adequate flow and causes only a slight edema. e) The sensitivity that increases with time, described by some investigators, must be proved quantitatively.

2) The curves of gradual responses in function of the logarithm of the doses are sigmoid, with two inflections placed approximately between the 20 and 80 % of action, this portion of the curve is a statistically significant straight line. The precision indexes obtained, show a good sensitivity of the preparation.

3) The vasoconstrictor action of L-adrenaline in the Laewen-Trendelenburg preparation is higher than that of L-noradrenaline. The relative potency of both hormones varies according to the dose used, this makes a parallel assay impossible The relative potency of noradrenaline to adrenaline for an ED 50 %, es equal to 2.9

4) Cocaine increases the action of both hormones, though its effects on noradrenaline is greater.

Action of LH and FSH on spermiation of the toad. by M. H. BURGOS AND B. A. HOUSSAY. *(Instituto de Biología y Medicina Experimental de Buenos Aires e Instituto de Histología y Embriología de la Facultad de Ciencias Médicas de Mendoza).*

With highly purified preparations obtained of mammals pituitaries, LH was more active and provoked spermiation with lower doses (0.15 mg) than FSH (1 mg). Only when preparations of FSH completely free of LH would be available, it could be decided if the spermiation produced by higher doses of FSH is due only to impurification by small quantities of LH.

Enzyme-substrate compounds with the potassium activated yeast aldehyde dehydrogenase. by A. O. M. STOPPANI AND C. MILSTEIN. *(Departamento of Bioquimica, Escuela de Medicina, Universidad de Buenos Aires).*

1) It has been studied the kinetics of the potassium activated yeast aldehyde dehydrogenase. 2) The experimental data fit the theory of an enzyme-acetaldehyde-DPN compound as responsible of the enzymic action. 3) There are two enzyme-acetaldehyde compounds: the first ($K_s = 9.0\ 10^{-5}M$) with one acetaldehyde equivalent per active center, is the catalytically active, whereas the second ($K_s = 0.8\ 10^{-3}M$) with two acetaldehyde equivalents per active center is not active. The value of the dissociation constants of the enzyme-DPN and enzyme-DPNH compounds are $1.0\ 10^{-6}M$ and $5.0\ 10^{-4}M$, respectively. K_s and K_s are independant of the coenzyme concentration and K_{DPN} is independant of acetaldehyde concentration.

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(2) WHITTEMBURY, G., RAMÍREZ, M., FERNÁNDEZ, J., MONGE, C.: *Acta physiol. lat. amer.*, 1955, *5*, 117.

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	Castellano	*Inglés*		*Castellano*	*Inglés*
metro	m	m	milisegundo	ms	msec
centímetro	cm	cm	litro	l	l
milímetro	mm	mm	centímetro cúbico	cm^3	cc
micrón	μ	μ	mililitro	ml	ml
milimicrón	$m\mu$	$m\mu$	kilogramo	kg	kg
o	o	o	gramo	g	gm
Angström	Å	Å	miligramo	mg	mg
microgramo	μg	μg	Miliequivalente	mEq	mEq
gama	γ	γ	Curie	C	C
hora	h	hr	Milicurie	MC	MC
minuto	m	min	Microcurie	μC	μC
segundo	s	sec	por ciento	%	%

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